

**MENSTRUAL MIGRAINE:
THE ROLE OF OESTROGEN**

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UNIVERSITY OF LONDON

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ABSTRACT

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ABSTRACT

Research into the association between migraine and menstruation has been hampered by lack of an agreed definition for 'menstrual' migraine. This thesis presents evidence for increased risk of migraine without aura on or between the two days before menstruation and the first three days of bleeding. Within individual women menstrual attacks differ from attacks at other times of the cycle, being longer and more severe. These findings led to the development of definitions for 'pure menstrual migraine' and 'menstrually-related migraine', which have subsequently been adopted by the International Headache Society.

Further research identified an inverse relationship between oestrogen and migraine incidence. The follicular phase oestrogen rise was associated with reduced risk of migraine; late luteal oestrogen 'withdrawal' at menstruation was associated with increased risk of migraine.

In order to counteract the luteal phase oestrogen drop and prevent menstrual attacks, oestrogen supplements were used from the luteal phase oestrogen peak (day -6) through to the early follicular rise of endogenous oestrogen (day +2). Identification of ovulation using a fertility monitor enabled prediction of menstruation and accurate timing of oestrogen supplements, despite a wide inter- and intra-individual range in cycle length. The results showed that use of oestrogen supplements was associated with a significant reduction in migraine days compared to placebo. However, the benefits were offset by delayed oestrogen 'withdrawal' migraine.

In women with migraine in the pill-free interval of combined hormonal contraceptives, there was a trend for oestrogen supplements to prevent 'menstrual' attacks, although the dose used was suboptimal.

These findings support the hypotheses that menstrual migraine is a discrete clinical entity and is associated with oestrogen 'withdrawal'. Further, oestrogen 'withdrawal' migraine can be independent of menstruation and independent of ovulation. Oestrogen 'withdrawal' migraine can be prevented with oestrogen supplements, although the optimal regime has yet to be established.

**"IN ANY INVESTIGATION INVOLVING HUMAN SUBJECTS THE
MOST DIFFICULT PART IS GETTING HOLD OF THEM"**

Hamilton M. BMJ 1965;2:1048-1051

This thesis is dedicated to all the women who took part in these research projects. Their interest and willingness to find the answers to the hypotheses has been as great as my own. The success of the research presented here is the result of their commitment to the demands made of them and adherence to the protocols, sometimes at great personal cost.

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ABBREVIATIONS

COCs	Combined oral contraceptives
CHCs	Combined hormonal contraceptives
DMPA	Depot medroxyprogesterone acetate
EE	Ethinylestradiol
E ₃ G	Oestrone-3-glucuronide
EMU	Early morning urine
FSH	Follicle stimulating hormone
GCP	Good clinical practice
HRT	Hormone replacement therapy
IHS	International Headache Society
LH	Luteinizing hormone
PdG	Pregnanediol 3-glucuronide
PFI	Pill-free interval
RR	Relative risk

LIST OF ORIGINAL PUBLICATIONS FORMING THE BASIS OF THIS THESIS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I MACGREGOR EA, CHIA H, VOHRAH RC & WILKINSON M (1990) Migraine and menstruation: a pilot study. *Cephalalgia* 10, 305-310.
- II MACGREGOR EA (1996) "Menstrual" migraine: towards a definition. *Cephalalgia* 16, 11-21.
- III MACGREGOR EA, IGARASHI H & WILKINSON M (1997) Headaches and hormones: subjective versus objective assessment. *Headache Quarterly* 8, 126-136.
- IV MACGREGOR EA & HACKSHAW A (2004) Prevalence of migraine on each day of the natural menstrual cycle. *Neurology* 63, 351-353.
- V MACGREGOR EA, FRITH A, ELLIS J, ASPINALL L & HACKSHAW A (2006a) Incidence of migraine relative to menstrual cycle phases of rising and falling estrogen. *Neurology* 67, 2154-2158.
- VI MACGREGOR EA, FRITH A, ELLIS J & ASPINALL L (2005) Predicting menstrual migraine with a home-use fertility monitor. *Neurology* 64, 561-563.
- VII MACGREGOR EA, FRITH A, ELLIS J, ASPINALL L & HACKSHAW A (2006b) Prevention of menstrual attacks of migraine: a double-blind placebo-controlled crossover study. *Neurology* 67, 2159–2163.
- VIII MACGREGOR EA & HACKSHAW A (2002) Prevention of migraine in the pill-free interval of combined oral contraceptives: a double-blind, placebo-controlled pilot study using natural oestrogen supplements. *J Fam Plann Reprod Health Care* 28, 27-31.

In addition, some unpublished data are presented.

AIMS OF THE THESIS

HYPOTHESES

1. Menstrual migraine is a discrete clinical entity
2. Menstrual migraine is associated with oestrogen 'withdrawal'
3. Menstrual migraine can be prevented with oestradiol supplements

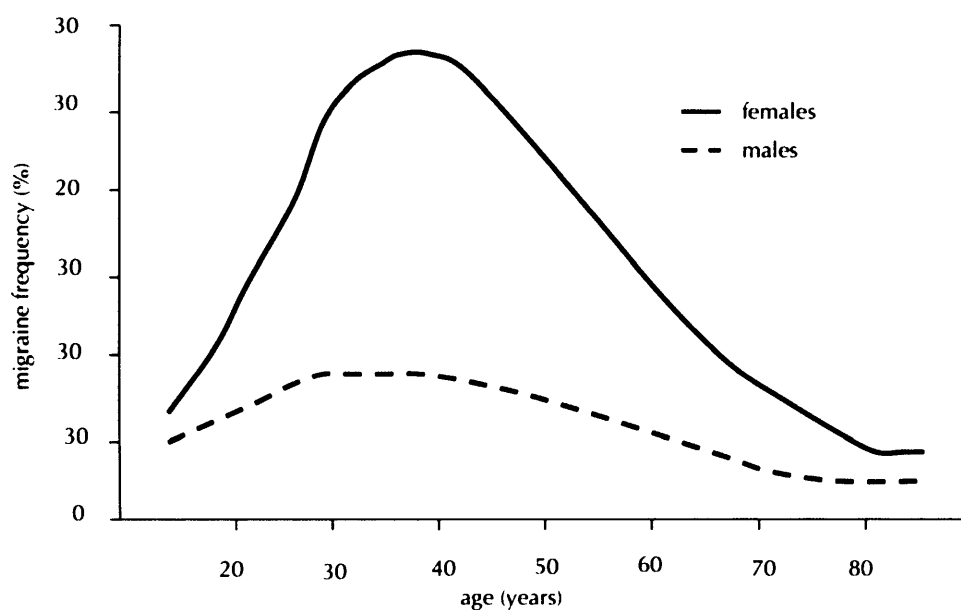
RESEARCH UNDERTAKEN TO TEST THE HYPOTHESES

1. Menstrual migraine is a discrete clinical entity
 - 1.1. Define menstrual migraine (PAPERS I, II, III, IV)
 - 1.2. Assess the clinical differences between menstrual migraine and non-menstrual attacks (PAPERS III, IV, V)
2. Menstrual migraine is associated with oestrogen 'withdrawal'
 - 2.1. Evidence for the association between oestrogen 'withdrawal' and migraine during the natural menstrual cycle (PAPER V)
 - 2.2. Evidence for the association between oestrogen 'withdrawal' and migraine during the hormone-free interval in women using combined hormonal contraceptives
3. Menstrual migraine can be prevented with oestradiol supplements
 - 3.1. Identify the optimal dose and timing of oestradiol supplements for prevention of menstrual migraine during the natural menstrual cycle (PAPER V)
 - 3.2. Identify a method to time the start of perimenstrual oestradiol supplements accurately (PAPER VI)
 - 3.3. Study the effect perimenstrual oestradiol supplements on the prevention of menstrual migraine during the natural menstrual cycle (PAPER VII)
 - 3.4. Study the effect oestradiol supplements on the prevention of migraine during the pill-free interval in women using combined oral contraceptives (PAPER VIII)

INTRODUCTION

PREVALENCE OF MIGRAINE

Headache is the most common neurological condition, with more than 90% of the population reporting headaches at some time in their lives.(Rasmussen, 1995) Migraine affects more than 14% (7.6% of men and 18.3% of women) of the UK population - over 6 million people - and is more prevalent than diabetes, epilepsy and asthma combined.(Steiner *et al.*, 2003) Prevalence of migraine varies with age, rising through early adult life and peaking during the most productive working years (Figure 1).



Adapted from: Lipton *et al.* Headache 2001; 41:646-657

FIGURE 1: ADJUSTED AGE-SPECIFIC PREVALENCE RATES BY SEX

THE BURDEN OF MIGRAINE

Migraine has been ranked by the World Health Organization as 19th among all diseases worldwide causing disability and 12th in women.(World Health Organization, 2001) Research shows that in the United Kingdom alone, an estimated 5.7 working days are lost per year for every working or student migraineur and each working day up to 90,000 people are absent from work or school as a result of migraine.(Steiner *et al.*, 2003)

Despite the high prevalence and disability, migraine is not seen as a public health problem; it is widely under diagnosed and under treated, both in children and adults.(Lipton *et al.*, 2003; Bigal *et al.*, 2006; Stovner *et al.*, 2007) It has been estimated that in the UK and the USA around two-thirds of people with migraine never consult their doctor, are not given a correct diagnosis, and treat attacks only with over-the-counter medication.(Lipton *et al.*, 2003) Many migraineurs would benefit from correct diagnosis and treatment with more specific drugs. Under-treatment – as well as causing unnecessary disability and suffering – is not economically cost-effective in terms of time lost from work and burden placed on the families of sufferers. More effective health care would alleviate much of the suffering and therefore reduce both the personal and financial costs of migraine.

MIGRAINE IN WOMEN

The burden of migraine particularly falls on women. Epidemiological studies confirm the clinical impression that migraine is a predominantly female disorder, affected by the changing hormonal environment during the reproductive years.

A study of 9059 children undertaken by Bille in 1955 showed that before puberty there was no sex difference in the prevalence of migraine; between the ages of 7 to 9 years migraine prevalence was approximately 2.5% in both boys and girls.(Bille, 1962) After the age of 11 there was an increasing female predominance, which became more marked in 13 to 15 year-olds. Notably, the median age at menarche is 12.8 years (range 9.1-17.7) in developed countries.(Speroff & Fritz, 2004)

By mid-life, migraine is three times more prevalent in women than in men.(Lipton *et al.*, 2001) Overall, studies suggest that by age 40 prevalence of migraine in

women is around 25%, compared with only 8% in men (Figure 2). (Rasmussen *et al.*, 1991; Scher *et al.*, 1999) In later life, migraine prevalence declines in both sexes although a female predominance remains.

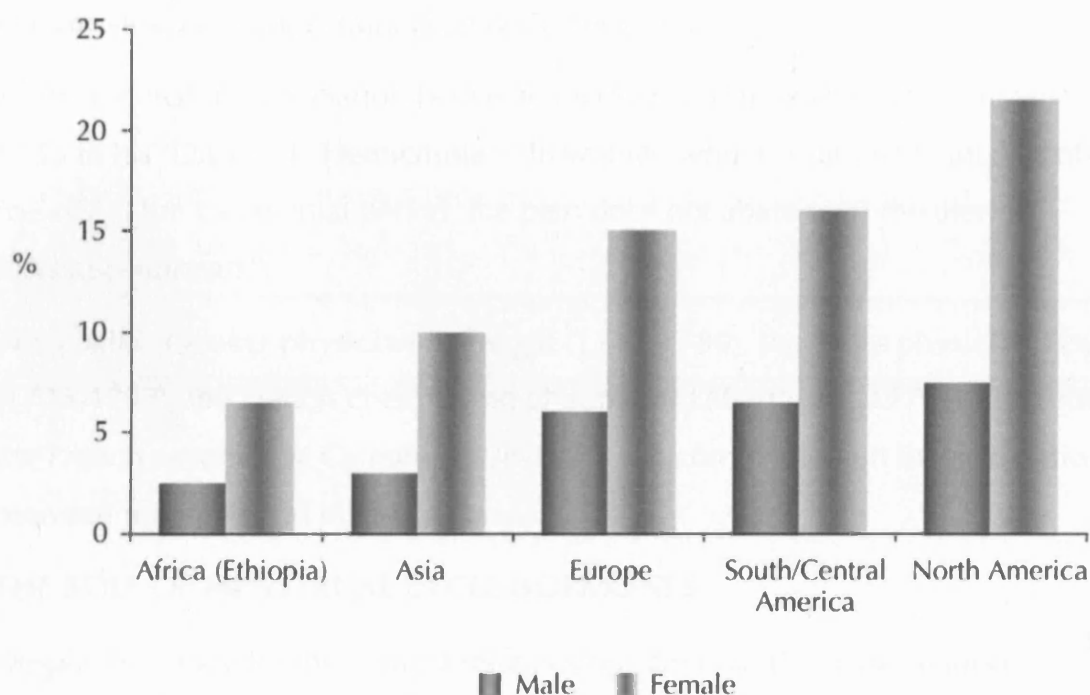


FIGURE 2: MIGRAINE PREVALENCE AT AGE 40 YEARS. BASED ON 18 POPULATIONS STUDIES THAT USED IHS CRITERIA. (SCHER *ET AL.*, 1998)

With regard to hormonal changes, ovarian function wanes around age 40 and the frequency of ovulation decreases, heralding the menopause. This stage of life, known as the climacteric, typically lasts around 10 years, centring on the mean age at menopause of 50.8 years.

The sex difference during the reproductive years is generally considered to result from the additional trigger of the menstrual cycle, an association that has long been recognised. Hippocrates noted that: "Shivering, lassitude and heaviness of

the head denotes the onset of menstruation".(Bronfen, 1998) The term 'hysteria' was used, which had physical rather than psychological connotations in historic times, simply meaning 'arising from the womb'. The recommended cure was marriage.

In 1666 Johannis van der Linden described a particularly severe case of one-sided headache with nausea and vomiting associated with menstruation in the Marchioness of Brandenburg.(Van der Linden, 1666)

Fordyce noted an association between migraine and the premenstruum, writing in 1758 in his 'Dissert de Hemicrania': "In women who are subject to attacks of this malady at the catamenial period, the pain does not abate until the uterine discharge appears".

The English Quaker physician Fothergill (1712-1780), the Swiss physician Tissot (1728-1797), the French chemist and pharmacist Labarraque (1777-1850), and the French psychiatrist Calmeil (1798-1895), all commented on the association between migraine and menstruation.

THE ROLE OF MENSTRUAL CYCLE HORMONES

Despite evidence for the clinical relationship, the underlying mechanisms remained elusive to researchers over the centuries. In 1873, Liveing noted that this variety of migraine was relieved by pregnancy with attacks "renewed again some time after delivery, occasionally with unusual severity; and this lends additional support to the general impression that there is some real connection between the attacks and the catamenial function."(Liveing, 1873)

The influence of menstrual cyclicity was suggested in epilepsy, 'paroxysmal insanity', asthma and various forms of neuralgia. Liveing asked: "How are we to interpret the facts; what is the character of the influence exerted and to what extent is it the cause of the malady?" In response, he quoted Dr Symonds 1858 'Gulstonion Lectures': "The menstrual function only acts as an exciting cause on an antecedent constitutional or neurotic susceptibility". Liveing also remarked that migraine could occur at monthly intervals in men and considered that the relationship of migraine to the catamenial period in women could be coincidental.

During the 20th Century, several hormonal treatments for migraine were attempted, with varying success. These included pituitary extracts containing gonadotrophins, human chorionic gonadotrophins extracted from the urine of pregnant women, ovarian oestrogens, progesterone, progestogens and testosterone.(Pardee, 1919; Blackie & Hossock, 1932; Thompson, 1932; Moffat, 1937; Singh & Singh, 1947; Moehlig, 1955; Hudgson *et al.*, 1967; Bradley *et al.*, 1968; Lundberg, 1969; Dalton, 1973; Greenblatt & Bruneteau, 1974)

The 1960s heralded the development of the contraceptive 'Pill', containing synthetic hormones ethinyloestradiol and progestogen. Reports of its negative effects on migraine abounded.(Whitty *et al.*, 1966; Sluglett & Lawson, 1967; Phillips, 1968; Larsson-Cohn & Lundberg, 1970; Pluvinae, 1970; Bickerstaff, 1975; Kudrow, 1975; Dalton, 1976; Ryan, 1978) In contrast, oestrogen replacement for migraine associated with ovarian failure showed benefit.(Chinn, 1968; Eichner, 1968; Chaudhuri & Chaudhuri, 1975)

With greater understanding of the hormones involved, more recent research has focused on the association between migraine and the declining levels of oestrogen and progesterone during the luteal phase of the menstrual cycle as well as withdrawal of ethinyloestradiol and progestogens in the hormone-free interval of combined hormonal contraceptives. However, research has been hampered by the lack of an accepted definition for menstrual migraine.

MENSTRUAL MIGRAINE

In 1988, the International Headache Society published the first classification and diagnostic criteria for headache disorders.(Headache Classification Committee of the International Headache Society, 1988) This classified most headaches but noted that there were no generally accepted criteria for so-called 'menstrual migraine'.

When I started working at the City of London Migraine Clinic in 1988, it was notable that many women reported that they had more migraine attacks around the time of menstruation. Menstrual attacks were clinically different from non-menstrual attacks, being more severe, lasting longer and less responsive to treatment.

A review of the literature at this time was unhelpful – the studies used numerous different definitions for 'menstrual' migraine and so were non-comparable.

In order to define the condition, I considered that the first step should be to study the incidence of migraine at different stages of the menstrual cycle. A definition could be based on the time of peak incidence. Once defined, research could focus on specific mechanisms that relate to the precise timing of increased incidence. Similarly, treatment trials could ensure that timing of treatment was targeted to the 'prone' time in a defined population of 'menstrual' migraine patients.

PART ONE:
MENSTRUAL MIGRAINE IS A DISCRETE CLINICAL ENTITY

1.1. DEFINING MENSTRUAL MIGRAINE

'Menstrual' migraine is a term commonly (mis)used by both patients and doctors without certainty of its existence.

In 1988, The International Headache Society published the first classification and diagnostic criteria that classified most headaches but not menstrual migraine. It stated:

'Migraine without aura may occur almost exclusively at a particular time of the menstrual cycle – so-called menstrual migraine. Generally accepted criteria for this entity are not available. It seems reasonable to demand that 90% of attacks should occur between two days before menses and the last day of menses, but further epidemiological knowledge is needed'.(Headache Classification Committee of the International Headache Society, 1988)

If menstrual migraine does exist as an entity, an accepted definition is essential to enable more specific research into its pathophysiology, and to develop more effective and specific treatment for this disabling condition. The definition should continue to be revised on the basis of further clinical research.

Correct classification of menstrual migraine is perhaps even more relevant as treatment becomes increasingly costly, as may be the case if triptans are more widely used for prophylaxis as well as acute therapy.

However, even before a definition can be developed, it is first necessary to confirm the existence of menstrual migraine as a discrete entity.

1.1.1. PAPER 1: MIGRAINE AND MENSTRUATION: A PILOT STUDY

OBJECTIVES

The objectives of this study were to determine the prevalence of migraine associated with menstruation in an outpatient population and to use the data to develop a preliminary definition for menstrual migraine.

METHODS

Patients and methods

Women attending the City of London Migraine Clinic were asked to keep a record of their migraine attacks and menstrual periods as a routine part of their treatment programme. During a 6-month period, diary cards from women with migraine with aura and/ or migraine without aura were collected at their follow-up visits, typically three months after their first appointment. Migraine was defined in accordance with the IHS criteria.(Headache Classification Committee of the International Headache Society, 1988)

The data were collated and analysed. Records kept by women using hormonal treatments such as hormonal contraception or hormone replacement therapy, or who were breastfeeding, were excluded from the data analyses. Migraine preceding and following day 1 (i.e. the first day of the bleeding) of each menstrual cycle were recorded on charts for individual women. In this way, headaches associated with each phase of the menstrual cycle could be easily identified for each woman. On the basis that the luteal phase is a relatively constant 14 days, migraine occurring at the time of ovulation would thus be expected around day -14 even in women who did not have a regular 28-day cycle.(Speroff & Fritz, 2004) The results were also summated on a single chart of all cycles for all women.

RESULTS

Over three hundred women were asked to keep records at their first visit to the clinic. Fifty-five women aged between 17 and 50 years (mean $35.8 \pm \text{SD } 9.0$ years) kept complete records of migraine attacks over three menstrual cycles and

were not using hormonal treatments. Eleven women had migraine *with* aura (20%) and 44 had migraine *without* aura (80%).

Figure 3 shows all migraine attacks recorded by the 55 women over three complete menstrual cycles. There was a marked increase in migraine incidence around the first day of menstruation (i.e. the start of the bleeding). There was no increased incidence at the estimated time of ovulation (day -14). Attacks recorded at each end of the chart are due to the variability in the length of menstrual cycles.

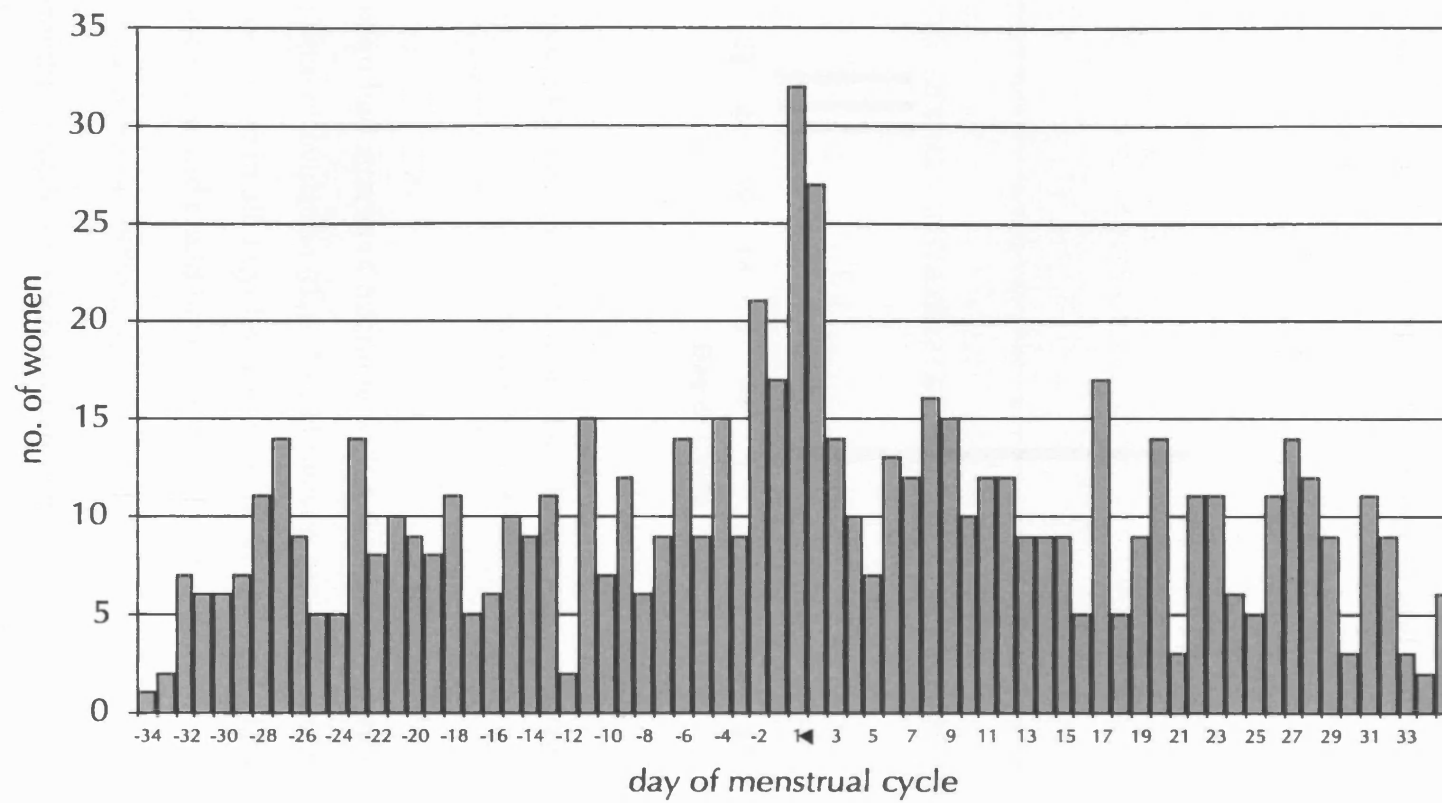


FIGURE 3: INCIDENCE OF MIGRAINE OVER THREE CYCLES IN 55 WOMEN

Six of the women (10.9%) had migraine attacks occurring only at the time of menstruation and at no other time. Of these, four (7.2%) had attacks in all 3 cycles recorded which occurred *only* between days -2 to +3 of the cycle (i.e. two days before menstruation and the first three days of bleeding). The other 2 patients had migraine attacks occurring also on day -4 and day +4.

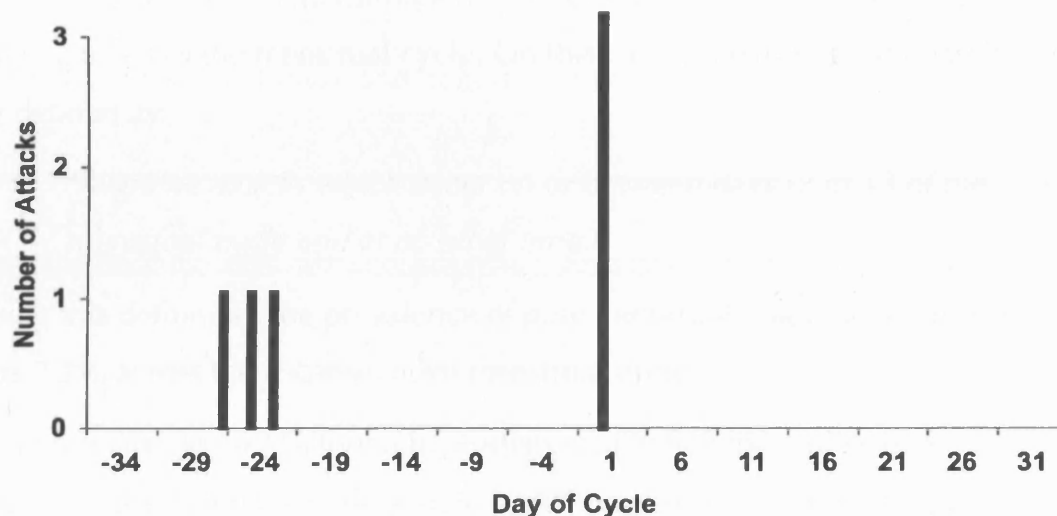


FIGURE 4: EXAMPLE OF INCIDENCE OF MIGRAINE OVER THREE CYCLES IN A WOMAN WITH PURE MENSTRUAL MIGRAINE

Only one woman had attacks of migraine within the -2 to +3 period as well as at the estimated time of ovulation (day -14). However, attacks associated with menstruation occurred in all 3 cycles, whereas the attack at ovulation was only recorded in one cycle and could have occurred by chance.

All 7 women had migraine *without* aura.

When the individual graphs for each woman were studied, 37 women (67.3%) had attacks during all stages of the menstrual cycle over the three cycles studied. Of these, 19 (34.5% of total) had an increased number of attacks within the period defined for 'menstrual' migraine; 18 (32.7%) had attacks throughout the cycle but with no increase in number during the defined period; 14 (25.5%) had

no attacks within the defined period during the three cycles. Of the women who had both migraine *with* and *without* aura and increased incidence of migraine between days -2 to +3, menstrual attacks were *without* aura.

DISCUSSION

In a study such as this it is difficult to overcome the problems of self-selection as only a small number of women were motivated enough to keep a clear record of all attacks and many charts were inadequately completed.

However, the effect of menstruation on migraine was most apparent between the days -2 to +3 of the menstrual cycle. On this basis pure menstrual migraine could be defined as:

'Migraine attacks which occur on or between days -2 to +3 of the menstrual cycle and at no other time.'

Using this definition, the prevalence of pure menstrual migraine in this population was 7.2% across three consecutive menstrual cycles.

This compares to 14.1% found by Epstein *et al.* when the replies to a questionnaire completed adequately by 92 women still menstruating were analysed and checked by interview. (Epstein *et al.*, 1975) These women claimed a regular relationship of their attacks of migraine to the menstrual cycle although this was not confirmed against prospective records. The original questionnaire was sent to all women over the age of 14 who had attended the Oxford Migraine Clinic over a 3-year period, so the replies were from a self-selected population.

Digre and Damasio found 8% of women with migraine had menstrual migraine using their definition of 'a common migraine that occurs during the week before or the week of menstruation, the women being headache free for the remainder of the cycle'. (Digre & Damasio, 1987) They also included women who they considered to have migraine at ovulation as well as during the menstrual flow. In the present study, there was no clear association between attacks of migraine and ovulation.

For the majority of women, migraine attacks could occur at any time of the month, as well as being more prevalent around the time of menstruation. On this basis 'menstrually-related migraine' could be defined as:

'Migraine attacks which occur regularly on or between days -2 to +3 of the menstrual cycle with additional attacks at other times of the cycle.'

Migraine attacks are triggered by several factors and menstruation must act as an additional trigger at that time. This may or may not be a specifically hormonal effect due to a direct action of the hormones or secondary to changes in the hypothalamic-pituitary axis. The effects on other biochemical pathways or even the effect of other trigger factors (dietary, sensory, stress, etc.) may also play a role. It would seem that there are only a few women for whom menstruation is the sole trigger of their attacks of migraine.

SUMMARY OF FINDINGS

Migraine was most prevalent on or between two days before the onset of menstruation and the first three days of bleeding (day 1 ± 2 days). Using this as a definition for menstrual migraine, four categories of women were identified:

1. Those with pure 'menstrual' migraine (7.2%);
2. Those with 'menstrually-related' migraine (34.5%);
3. Those whose attacks occur throughout the menstrual cycle but with no increase at the time of menstruation (32.7%);
4. Those whose attacks do not occur at the time of menstruation (25.5%).

Menstrual attacks were typically *without* aura, even in women who also had migraine *with* aura. No link between migraine and ovulation was identified.

FURTHER WORK

It was felt that further studies are required to identify the specific groups and to analyse any further differences between the groups in the light of previous studies. For example, patients identified as having 'menstrual' migraine need to be studied for a much longer period to assess whether or not the temporal relationship of attacks to menstruation continues. Also the finding that all four women with pure menstrual migraine had migraine *without* aura needs to be corroborated with larger study numbers. The effect of other trigger factors needs to be studied - a greater link with menstruation may be found by removing as many triggers as

possible (e.g. excluding dietary triggers, eating regular meals, reducing caffeine intake, etc.).

A larger series is needed to enable a firm statement to be made about the duration of increased susceptibility. This susceptibility could then be correlated with known hormonal changes and the effect of ovulation, which may have been masked in such a small group as mentioned above.

The identification of the four distinct groups may well have an implication for the treatment of migraine in women. For example, it is most likely that hormonal treatment will be effective for the group with pure menstrual migraine and may help the group with menstrually-related migraine. It is unlikely that hormonal treatment will help those whose attacks are not related to menstruation. Further controlled studies will need to be undertaken to support this hypothesis.

1.1.2. PAPER II: DEFINING MENSTRUAL MIGRAINE: A REVIEW

The results presented in Paper I provide evidence for the existence of menstrual migraine, suggesting that menstrual attacks are most likely to be migraine without aura and occur on or between days -2 to +3 of the cycle.

In considering a definition, a review of published definitions was undertaken.

Figure 5 shows the different timings for attacks described as 'menstrual' migraine. The interval during which menstrual migraine attacks could occur ranged from a 3-day interval to a 15-day interval.(O'Dea & Davis, 1990; Lichten *et al.*, 1991) The timing for these intervals varies. In some definitions attacks are premenstrual (before the onset of menstruation) or perimenstrual (before and during menstruation); others define attacks during menstruation only or include ovulation.

Table 1 shows that definitions with similar time intervals may still differ from one another. Some researchers included attacks at any stage of the cycle provided that attacks occurred regularly within the specified interval for menstrual migraine. For others, the definition was only met when attacks fall solely within the specified interval on every occasion with no attacks at other times of the cycle. It is important to distinguish between the two since a pure hormonal mechanism is likely to be involved only in women with pure menstrual migraine. In contrast, additional non-hormonal factors will be acting in women with additional non-menstrual attacks. If studies are to uncover the mechanisms responsible for menstrual migraine, it is necessary to study women in whom hormonal events are the sole association.

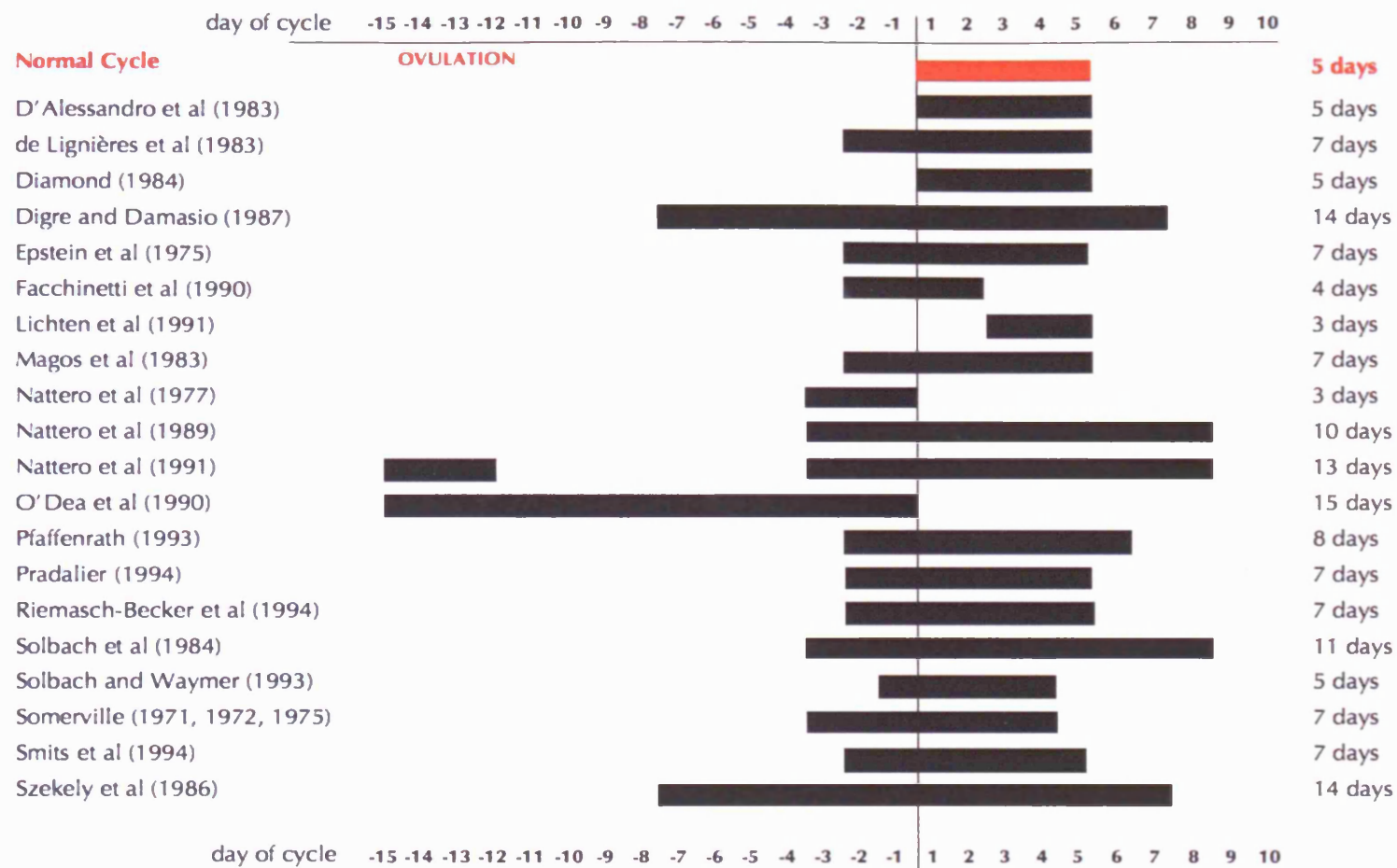


FIGURE 5: VARIATION OF DEFINITIONS FOR 'MENSTRUAL' MIGRAINE BASED ON AVERAGE DURATION OF FIVE DAYS OF MENSTRUAL BLEEDING

AUTHORS	PREDEFINED OR RETROSPECTIVE DEFINITION	TIGHTEST DEFINITION	TYPE OF MIGRAINE	DOES DEFINITION ALLOW ATTACKS AT OTHER TIMES?
Benedetto et al (1987)	Predefined	Migraine that occurs only prior to, during, or at the end of menstruation and/or at the time of ovulation	Common or classical (Ad Hoc)	No
D'Alessandro et al (1983)	Predefined	Migraine only or predominantly during menses	Not specified	Yes
Dalton (1973)	Clinical assessment	Attacks of severe disabling headaches always recurring at the same phase of each menstrual cycle...highest daily incidence was on the two days immediately preceding menstruation	Not specified	Yes N.B. Included women taking oral contraception
de Lignières et al (1986)	Predefined	Common migraine occurring exclusively just before or during menstruation (day -2 to last day of menses)	Common	No
Dennerstein et al (1988)	Predefined	Regular migraine in the paramenstruum	Any	Yes
Diamond (1984)	Not specified	Exclusively at menses	Not specified	Not specified
Digre and Damasio (1987)	Not specified	Common migraine that only occurs during the week before or the week of menstruation; the woman is headache free for the remainder of the cycle	Common (Ad Hoc)	No
Epstein et al (1975)	Predefined	Migraine confined to just before or during the period of blood loss	Not specified	No
Facchinetti et al (1990)	Predefined	Migraine attacks normally starting -2 and -1 days before menses, lasting until 2nd day of the menstrual flow	Any (ad hoc)	Yes

Note: 'common' and 'classical' migraine was diagnosed according the Ad Hoc criteria for migraine, which predates the IHS classification.(Ad Hoc Committee of the NIH, 1962) The terms are equivalent to 'migraine without aura' and 'migraine with aura' respectively

TABLE 1: DEFINITIONS FOR MENSTRUAL MIGRAINE

Facchinetti et al (1993)	Predefined	Migraine attacks that occurred between day -2 and day +3 in relation to the onset of the menstrual flow	Common (IHS)	?No
Facchinetti et al (1995)	Predefined	Migraine attacks occurring -3 to +5 days relative to the start of menstruation	Not specified	Not specified
Gallagher (1989)	Predefined	A migraine attack almost exclusively associated with the menstrual cycle	Not specified	Not specified
Gross et al (1995)	Predefined	A retrospective diagnosis of $\geq 80\%$ of attacks occurring during the menstrual window (day -3 to +5) in the past 6 months	Migraine \pm aura (IHS)	Yes
Lichten et al (1991)	Predefined	'Hormonal migraine': migraines during the luteal phase of the menstrual cycle - most typically within 7-10 days prior to menses: includes 'menstrual migraine': headache during menstrual phase only - after 2nd full day of menstruation	Any (Ad Hoc)	Not specified
Lundberg (1986)	Not specified	The migraine attack is often associated with a particular day of the menstrual cycle that is characteristic for each woman. It commonly occurs just before the menstrual period, often together with the premenstrual syndrome...Migraine attacks may also occur at the time of ovulation and during or immediately after menstruation	Not specified	Not specified
MacGregor et al (1990)	Clinical assessment	Migraine attacks which regularly occur on or between days -2 and +3 of the menstrual cycle and at no other time	Common (IHS)	No
Magos et al (1983)	Predefined	Regular attacks immediately before or during menstruation	Classical and common (WFN)	Not specified

Note: 'common' and 'classical' migraine was diagnosed according the Ad Hoc criteria for migraine, which predates the IHS classification. (Ad Hoc Committee of the NIH, 1962) The terms are equivalent to 'migraine without aura' and 'migraine with aura' respectively

TABLE 1: DEFINITIONS FOR MENSTRUAL MIGRAINE

Martignoni et al (1987)	Review article	Menstrual migraine most commonly appears just prior to the onset of the period (premenstrual migraine) but it may also occur during or after the actual flow. Sometimes it also occurs at the time of ovulation	Common	Not specified
Mathew (1986)		No definition	Common	Not specified
Nattero et al (1977)	Predefined	Migraine a few days before the onset of menstruation	Not specified	Not specified
Nattero et al (1989)	Predefined	Onset of the migraine crisis exclusively prior to, during, or at the end of menstruation	Classical or common (Ad Hoc)	No
Nattero et al (1991)	Predefined	Migraine exclusively prior to, during, or at the end of menstruation and....also at the time of ovulation	Any	No
O'Dea et al (1990)	Predefined	During the luteal phase of the menstrual cycle (between ovulation and menstruation)...associated with the premenstruum distinct from sporadic migraine	Not specified	Not specified
Pfaffenrath (1993)	Predefined	Migraine attacks occurring exclusively between 2 days prior to onset and first day after the offset of the menses	Not specified	No
Pradalier (1994)	Predefined	IHS definition: 90 per cent of attacks occur between 2 days before menses and the last day of menses	?Migraine without aura (IHS)	Yes (10%)
Riomasch-Becker (1994)	Predefined	Migraine onset between 2 days before or during menstruation	Not specified	Yes
Sances et al (1989)	Predefined	Migraine attacks linked with menstrual cyclicity	Not specified	Not specified
Sances et al (1991)	Predefined	A form of periodical headache that often fails to improve with usual prophylactic treatments	Not specified	Not specified
Schoenen (1985)	Predefined	Exclusively perimenstrual	Not specified	No

Note: 'common' and 'classical' migraine was diagnosed according the Ad Hoc criteria for migraine, which predates the IHS classification.(Ad Hoc Committee of the NIH, 1962) The terms are equivalent to 'migraine without aura' and 'migraine with aura' respectively

TABLE 1: DEFINITIONS FOR MENSTRUAL MIGRAINE

Solbach et al (1984)	Predefined	Any migraine headache which occurs 3 days prior to menstrual flow, during time of flow, or 3 days following	Predominantly classical or common	Not specified
Solbach and Waymer (1993)	Predefined	Attacks during a 5 day period from 1 day before start of menstrual flow through the first 4 days of flow	Migraine with or without aura (IHS)	Yes
Somerville (1971,1972,1975)	Predefined	One predictable attack of migraine per month always confined to premenstrual or menstrual phases	Severe and throbbing headache, lasting several hours accompanied by nausea and photophobia \pm visual or sensory symptoms	No
Szekely et al (1986)	Predefined	Headache occurring regularly within plus or minus one week of day one of menstruation	Headache	Not specified
Waters and O'Connor (1971)	Clinical assessment	Incidence of migraine attacks twice as frequent during menstruation and non migraine headaches more than twice as frequent during menstruation - highest incidence during 1st few days of menstruation.	Unilateral headache, warning before headache, accompanying nausea	Yes

Note: 'common' and 'classical' migraine was diagnosed according the Ad Hoc criteria for migraine, which predates the IHS classification.(Ad Hoc Committee of the NIH, 1962) The terms are equivalent to 'migraine without aura' and 'migraine with aura' respectively

TABLE 1: DEFINITIONS FOR MENSTRUAL MIGRAINE

Table 1 also shows the type of migraine for each definition. In the majority of cases any type of migraine is allowed, or the type of migraine is not specified.

Stricter definitions confine attacks to those of migraine without aura but again, the time interval ranges enormously from approximately 7 days to approximately 14 days.(de Lignieres *et al.*, 1986; Digre & Damasio, 1987)

In some cases the definition is very loose, e.g. 'migraine attacks exclusively prior to, during, or at the end of menstruation, and... also at the time of ovulation'; 'a migraine attack almost exclusively associated with the menstrual cycle'; 'a form of periodical headache that often fails to improve with usual prophylactic treatment'.(Gallagher, 1989; Nattero *et al.*, 1991; Sances *et al.*, 1991) Such definitions are unhelpful for clinical practice or further research.

The strictest definition for 'menstrual' migraine was the one used by de Lignières *et al.* and Smits *et al.*: 'attacks of migraine without aura occurring not earlier than two days before menstruation and not later than the last day of the menses with no other headache attacks during the rest of the cycle.' The attacks had to be regular, always occurring at the same time in the menstrual period, and to have occurred during each of the 12 cycles preceding the study. Further, the duration of menstrual cycles had to be consistent, not differing in length by more than two days for all 12 cycles.(de Lignieres *et al.*, 1986; Smits *et al.*, 1994)

TOWARDS A DEFINITION FOR MENSTRUAL MIGRAINE

If a definition is to be developed, several points need to be considered:

Did the researchers diagnose migraine accurately?

Many of the studies were undertaken before the introduction of the IHS diagnostic criteria and migraine may not have been diagnosed accurately. Correct diagnosis of migraine is essential - several studies have noted an increase in non-migraine headaches perimenstrually, particularly in women who fulfil criteria for the premenstrual syndrome.(Waters & O'Connor, 1971; Metcalf, 1988; Keenan & Lindamer, 1992)

At what stage of the menstrual cycle is migraine most likely to occur and what type of migraine?

The majority of studies using a definition for menstrual migraine were undertaken to assess different treatments. The definition was predetermined without prior evaluation of the natural incidence of migraine across the menstrual cycle.

The results of Paper I suggest that migraine incidence peaks on or between day -2 and day +3 of the cycle. Further, menstrual attacks were *without* aura.

In a study undertaken in a specialist clinic, Dalton studied 52 women with menstrual migraine, defined as attacks of severe disabling headaches, always recurring at the same phase of each menstrual cycle. (Dalton, 1973) Of 512 attacks recorded, 36% occurred during the four days immediately before menstruation and 30% occurred during the first four days of menstruation. The peak incidence was two days immediately before the onset of menstruation. There was no association with ovulation.

In a community based study Waters and O'Connor, analysed data from 117 women aged between 20 and 64 years. (Waters & O'Connor, 1971) In menstruating women, the highest incidence of migraine was reported during the first few days of menstruation. Of 42 women whose diary cards were analysed over two menstrual cycles, the incidence of migraine was about twice as frequent during menstruation compared to the rest of the cycle. There was no association with ovulation.

Johannes *et al.* studied a group of women who were participating in a large population-based prevalence survey of headache. (Johannes *et al.*, 1995) Seventy-four women aged between 22 and 29 kept diary records of all their headaches for up to 16 weeks. The authors noted a 60% increase in migraine *without* aura during the first three days of menstruation. However, they did not analyse severity. All women in the study had experienced at least one attack of migraine with aura during their lifetime. They were required to have at least two migraine attacks per month, thus the study included women for whom menstruation was not the sole trigger. No increase in any headache type was noted during the premenstrual phase or in relation to the estimated time of ovulation; however, 22 of the 74 subjects (30%) were using oral contraception during the study period.

Although the investigators stated that this was not found to be a significant covariant, the mechanism of attacks linked to the normal hormonal cycle may differ from attacks linked to the withdrawal bleed associated with combined oral contraceptive use.

In a population-based diary card study Stewart et al examined data from 81 women each contributing a mean of 89.1 diary days (total 7219 days). (Stewart et al., 2000) They found migraine *without* aura was more likely to occur two days before the onset of menstruation and on the first two days of menstruation. There was no association between menstruation and migraine *with* aura. They did not state whether or not any women were taking oral contraceptives.

Taken together, these studies confirm the timing of increased incidence of migraine on or between day -2 to +3 and provide further evidence that such attacks are *without* aura.

Can menstrual migraine be diagnosed subjectively or is objective diary data required?

Few studies paid attention to documented evidence of 'menstrual' migraine, usually relying on the patient history, or at most assessing only one or two months of diary cards. Patients' self-assessment of menstrual migraine is unreliable, an example being women saying that they only get migraine with their periods when their diary cards show attacks occurring at any stage of the menstrual cycle. Patients often report that their attacks occur "when I ovulate" - despite the fact that they are taking the combined hormonal contraceptives, which inhibit ovulation. Therefore studies obtaining data by questionnaire only, relying on subjective reporting of a link between migraine and menstruation, will lead to an overestimation of the association.

This concern was confirmed by the results from a prospective trial of oral sumatriptan 100mg in the treatment of menstrual migraine. (Dowson et al., 2005) Women who stated that more than 80% of their attacks occurred between -3 to +5 days from the onset of menstruation participated in a double-blind, placebo-controlled crossover study, conducted over four menstrual cycles. Prospective diaries reviewed at the end of the study revealed that only 11% of participants met the inclusion criteria.

The importance of keeping accurate diary information must be stressed to the patient. Waters and O'Connor stated: 'All 117 women who were examined clinically made some attempt to keep diaries of their headaches but the degree of co-operation was extremely variable'.(Waters & O'Connor, 1971) In Paper I presented above, more than 300 women were asked to keep records for three months, but only 55 kept diary cards that were complete and suitable for analysis.

Should attacks associated with 'periods' resulting from withdrawal of exogenous hormones (e.g. combined hormonal contraceptives or cyclical hormone replacement therapy (HRT)) be included in the definition?

The progestogen withdrawal bleed occurring during the hormone-free interval of combined hormonal contraceptives or cyclical HRT is not the same as a menstrual bleed resulting from the ovarian cycle, although both are often described as 'menstrual'. The endogenous menstrual cycle is the result of complex hormonal changes in the hypothalamic-pituitary-ovarian axis leading to ovulation. Menstruation follows the decline in hormones towards the end of the cycle. In contrast, ovulation is suppressed by use of combined hormonal contraceptives. This is important when considering whether the migraine is associated solely with a menstrual 'bleed' or by hormonal changes, as the mechanism(s) of migraine may be different in these distinct cases even if the timing of attacks is the same. Research should clearly separate these subpopulations in order to account for potential differences in pathophysiology.

How many cycles of data should be collected to confirm the diagnosis?

An additional factor is the number of cycles over which these attacks should occur. Clearly, a woman who has exclusive attacks in the defined interval over six cycles is more likely to have menstrual migraine than a woman who only has half her attacks occurring during the defined interval over fewer cycles. However, not all women can provide complete diary data for six cycles in advance of a clinical trial. A minimum of three cycles of data is likely to provide adequate information.

SUMMARY OF FINDINGS

A review of the literature revealed that there is no consensus for a definition for menstrual migraine. Researchers have used a number of different interpretations for the term, making it difficult to compare the data. The review confirms the findings in Paper I, that the incidence of migraine increases on or between day -2 and day +3 of the menstrual cycle and that menstrual attacks are *without* aura, even in women who have migraine *with* aura. Since attacks at the time of menstruation could be a chance occurrence, a definite association should be confirmed over several cycles. Balancing the need for accurate data against the inconvenience of keeping diaries, it is reasonable to suggest that menstrual attacks occurring in at least two out of three of menstrual cycles is sufficient evidence to confirm the association.

1.1.3. PAPER III: HEADACHES AND HORMONES: SUBJECTIVE VERSUS OBJECTIVE ASSESSMENT

Having identified that migraine is more prevalent around day 1 of menstruation, a study was undertaken to assess the relationship that women consider exists between their migraine attacks and the menstrual cycle.

The subjective account was compared to a prospective record kept by the women of their migraine attacks and menstrual cycle. The hypothesis was that self-assessment is unreliable and will lead to an overestimation of prevalence of menstrual migraine.

OBJECTIVES

The objective of this study was to compare the subjective assessment of the effects of menstruation on headaches and migraine against objective diary card data.

PATIENTS AND METHODS

One doctor interviewed a consecutive series of 100 women diagnosed with migraine with aura and/or migraine without aura when they attended the City of London Migraine Clinic. The women were asked specific questions regarding the effect of menstruation on their attacks of migraine and other headaches.

Based on the results of this questionnaire, the women were then divided into two groups:

Group 1: Those who thought that their attacks of migraine were related to menstruation;

Group 2: Those who did not think there was any relationship between migraine and menstruation.

All menstruating women were asked to keep a record of their migraine attacks and menstrual cycles over the following three menstrual cycles. In this way their initial self-assessment of migraine attacks associated with menstruation could be compared with a prospective record.

For each woman, the number of migraine attacks that occurred on each day of the three menstrual cycles were recorded on a chart. As in Paper I, attacks were

charted both preceding and following day 1 of the menstrual cycle (day 1 = the first day of bleeding). In this way the relationship of migraine attacks to each phase of the menstrual cycle could be studied. Attacks around the estimated time of ovulation could be identified around day -14, even if the cycles were irregular, as the luteal phase is relatively constant. (Speroff et al, 1989)

RESULTS

Of the 100 women interviewed, 22 (age 38.9+/- SD 12.9) had migraine *with* aura and 78 (age 38.0 +/- SD 11.7) had migraine *without* aura.

Eighty-four women were still menstruating and were not using hormonal treatment. Eleven women were post menopausal and 5 had had an hysterectomy with conservation of the ovaries.

Self-assessment

Of the menstruating women: 42 women (7 migraine *with* aura (16.7%), 35 migraine *without* aura (83.3%)) thought that their migraine attacks were related to menstruation (Group One); 41 women (11 migraine with aura (26.8%), 30 migraine without aura (73.2%)) did not think that their migraine attacks were related to menstruation (Group Two); one woman was uncertain of the presence of any link between migraine and menstruation.

Sixty-five women reported headaches of any kind (including migraine) occurring around the time of menstruation, all 42 (100%) in Group One and 23 (56.1%) in Group Two. One woman (2.4%) in Group One reported non-migraine headaches compared to 8 of the 23 women (34.8%) in Group Two. Nine women (21.4%) in Group One reported an increase in migraine at menstruation compared to only 2 women (8.7%) in Group Two.

Migraine and menstruation diary

Twenty women (24.1%) kept an accurate record over three consecutive menstrual cycles, 16 (2 migraine *with* aura, 14 migraine *without* aura) from Group One and 4 (all migraine *without* aura) from Group Two.

A marked increase in migraine was noted to cluster around day 1 of the cycle (first day of bleeding). There was no increase in incidence of migraine at the

expected time of ovulation (day -14).

Pure menstrual migraine was defined as 'migraine attacks which occur regularly on or between days -2 to +3 of the menstrual cycle and at no other time', as proposed in Paper I.(MacGregor *et al.*, 1990) Only three women (15%) fulfilled these criteria, all from Group One. All three women had migraine *without* aura.

'Menstrually-related' migraine was defined as 'migraine attacks which occur regularly on or between days -2 to +3 of the menstrual cycle with additional attacks at other times of the cycle'.(MacGregor *et al.*, 1990) Three women (15.0%) fulfilled these criteria and were all from Group One. All had migraine *without* aura.

The remaining ten women from Group One (8 migraine *without* aura, 2 migraine *with* aura) and one woman from Group Two, had attacks throughout the menstrual cycle with no increase at the time of menstruation. None of the other three women in Group Two had any attacks on or between days -2 and +3 over all three cycles.

Most women from Group One had been quite specific in the questionnaire regarding the timing of their attacks e.g. "always 5 days before my period", "only during my period". This relationship was not borne out by the diaries of the 13 women from Group One who did not have menstrual migraine but was accurate for the three women with pure menstrual migraine.

DISCUSSION

The findings of this study support the results of Paper I. They emphasise the requirement of definitive criteria for 'menstrual' migraine and 'menstrually-related' migraine and the need for prospective records to make the diagnosis, rather than relying on subjective reporting of a menstrual association.

Previous studies have reported that between 26-60% of women with migraine relate a periodicity of their headaches to their menstrual cycles.(Epstein *et al.*, 1975; Edelson, 1985; Nattero, 1982; Lance, 1982; Dalton, 1976) This compares with our finding of 50.6%. Epstein *et al.* (1975) defined true 'menstrual' migraine as attacks confined to just before or during the period of blood loss which occurred in 14% of menstruating women, with a further 12% having additional

attacks at other times. Our findings of 15% and 15% respectively were similar. Women who completed our prospective study were a self-selected group who knew that a connection between migraine and menstruation was being studied. Unfortunately data in this part of the study was highly selected as so few women kept complete diary cards for the three months. Our previous study on a larger number of women who kept records as part of their routine treatment showed that 7.2% of women had 'menstrual' migraine and 34.5% had 'menstrually-related' migraine. (MacGregor *et al.*, 1990)

SUMMARY OF FINDINGS

In this study the subjective link between migraine and menstruation as reported by women questioned often failed to match prospective records of migraine and menstruation. This discrepancy casts considerable doubt on some previous published research in which the diagnosis of 'menstrual' migraine was based upon subjective history or retrospective questionnaires. Information obtained at interview is of limited value in identifying women in whom a definite link between migraine and menstruation exists, although this study suggests that it does correctly identify women in whom there is no such link.

Suggested definitions for 'menstrual' migraine and 'menstrually-related' migraine are based on the clinical observation that women have more migraine attacks on or between days -2 to +3 of the cycle. Universally agreed criteria are necessary for the comparison of studies and specific treatment regimes.

These findings confirm that the terms pure menstrual migraine and menstrually-related migraine should only be used when strict criteria are fulfilled, enabling future studies to explore the pathophysiology of 'menstrual' migraine with fewer confounding triggers. Such criteria should also be met before specific hormonal treatment is considered.

1.2. ASSESSING THE CLINICAL DIFFERENCES BETWEEN MENSTRUAL MIGRAINE AND NON-MENSTRUAL ATTACKS

If menstrual migraine is distinct entity, there should be identifiable characteristics that distinguish such attacks from other types of migraine. Although it is generally considered that attacks of menstrual migraine are more severe and less responsive to treatment compared to non-menstrual attacks, there are limited data to support this clinical observation. Most studies have retrospectively analysed data from large clinical trials of acute treatment with triptans, comparing all attacks occurring in association with menstruation with non-menstrual attacks. Very few studies have focused specifically on women diagnosed with pure menstrual or menstrually-related migraine.

1.2.1. PAPER IV: PREVALENCE OF MIGRAINE ON EACH DAY OF THE NATURAL MENSTRUAL CYCLE

This study was undertaken to assess the association between migraine and menstruation over a larger number of cycles, in a larger number of women, in whom an accurate diagnosis of migraine has been made, than has previously been studied. Further, the data were subjected to a within-woman analysis rather than the usual between-women comparisons.

OBJECTIVES

The objectives of this study were to assess when migraine was most likely to occur during the menstrual cycle and if migraine at the time of menstruation differs in severity and the presence or absence of nausea or vomiting versus attacks at other times of the cycle.

METHODS

Women with migraine attending the City of London Migraine Clinic kept diary cards as part of their routine management. On the diary cards both headaches and migraine were recorded, together with severity (mild, moderate, or severe), presence of aura and nausea or vomiting.

Fully completed diary cards recording migraine attacks and menstruation over a minimum of three menstrual cycles in women who had migraine with and/or without aura were analysed.

A database was designed specifically for the study using Microsoft Access. One individual entered all the data, in order to ensure consistency of interpretation. Based on information from each woman's diary cards it was judged whether she suffered a headache or a migraine. Where there was any doubt about the nature of any headache episode, we assessed the accompanying symptoms or contacted the patient directly for clarification.

Because it was not known when in the cycle each woman began her diary, migraine attacks occurring before the first recorded period were not included in the analysis. Also, the date of the last period for each woman was not recorded so it was assumed that the length of the last recorded cycle would be 28 days.

Migraine attacks occurring more than 23 days from the last known period date were excluded in order to avoid including migraine attacks in the last cycle which may have occurred in the 5 days before the following period.

DEFINITIONS

The following definitions were used for the analysis:

- The pre-menstruation interval: the five days immediately before the first day of menstruation (days -5 to -1).
- The post-menstruation interval: the first day of menstruation and the five days immediately afterwards (days 1 to 6).
- 'All other times' included the days that did not fall within either the pre- or post-menstruation interval, i.e., all days of the cycle other than day 1 of menstruation \pm 5 days.

Additional analyses were based on the two days before menstruation (days -1 and -2) and the first day of menstruation plus subsequent two days of bleeding (days +1 to +3).

ANALYSES

Migraine was counted as each day on which the woman reported migraine. For each woman the risk of migraine during the pre-menstruation and during the post-menstruation intervals was compared to the risk of migraine at all other times of her cycle and the relative risk calculated.

Since diary data for the final cycle for most women was incomplete, a statistical model was developed to handle the data consistently. This is illustrated in Figure 6, which shows how each day was determined to be pre- or post menstruation or all other times for one woman who provided a diary for three consecutive cycles, with an incomplete third cycle. The final cycle was assumed to last 28 days, on the basis of mean duration, although it is recognised that there is marked individual variation. (Speroff & Fritz, 2004)

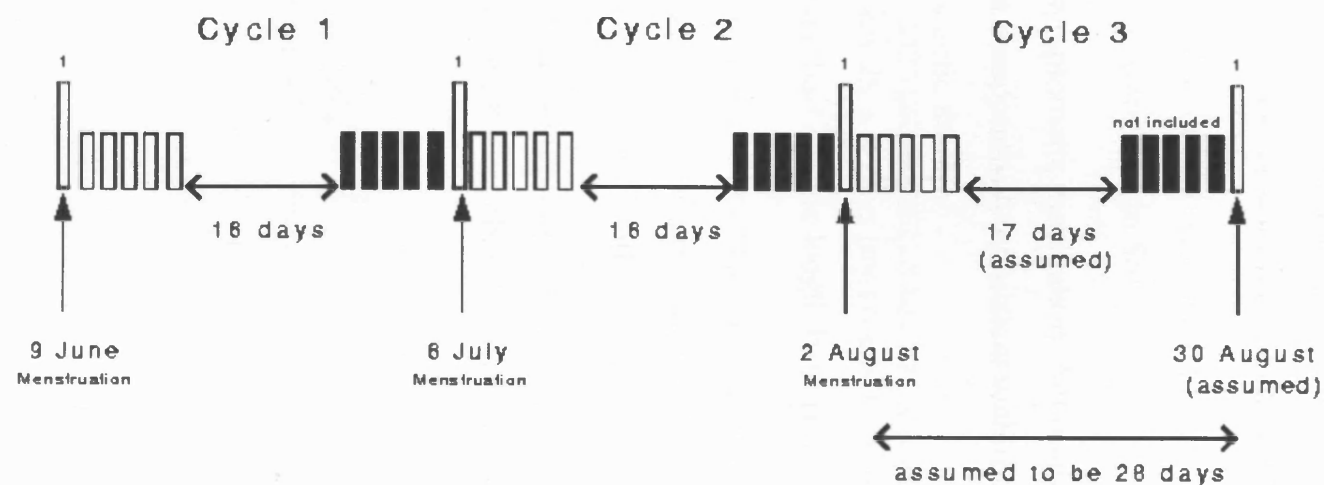
In this example, over the three recorded cycles, there were two known pre-menstruation intervals from day -5 to day -1 (of 10 days in total) during which there were two migraine attacks, three known post-menstruation intervals from



day +1 to day +6 (of 18 days in total) during which there were nine migraine attacks, and 49 days at all other times during which there were 11 migraine attacks. For this woman the relative risk of having a migraine attack in the pre-menstruation interval was 0.89 ($2/10 \div 11/49$). The relative risk of having an attack in the post-menstruation period was 2.23 ($9/18 \div 11/49$). This approach was, therefore, a within-woman comparison and would allow for her own migraine pattern during the course of her cycles.

If there were no migraine days for both the pre-menstruation interval and at other times (or both the post-menstruation interval and at other times) a relative risk was not calculated.

Relative risks were pooled across all women using an exact method which allows for small occurrences, providing a 95% confidence interval and p-value. (Martin & Austin, 2000)



Pre-menstrual interval = total number of migraine days out of 10 days (5+5)
(5 days before menstruation, solid bars)

Post-menstrual interval= total number of migraine days out of 18 days (6+6+6)
(on & 5 days after menstruation, open bars)

All other times= total number of migraine days out of 49 all other days (18+18+17)

FIGURE 6: EXAMPLE USING DATA FROM ONE WOMAN TO SHOW HOW DATA FROM THE FINAL CYCLE WERE ANALYSED

RESULTS

Diary cards from 165 women were reviewed. Six women were excluded because each had data for one cycle only and four women were excluded because the data were not from consecutive cycles. The analysis presented here is thus based on 155 women. There was a total of 693 cycles; 17% of women provided data for 2 cycles, 37% for 3 cycles, 16% for 4 cycles and 30% for 5 or more cycles.

Of the 155 women, date of birth was available for 143. The median age of these women was 44 years (range 15 to 58).

All women used symptomatic medication. Although this varied between individuals, it was comparatively consistent within individuals. Only six women were using prophylactic therapy.

The cycle length was 29 ± 4 days (mean \pm SD). Although the range was 19-44 days, 95% of women had a cycle length between 21 and 37 days.

Severity of migraine and whether there was nausea or vomiting was only recorded by 81 women: 33% of women (27/81) reported migraine with vomiting at some point, and 91% (74/81) with nausea.

Table 2 shows the total occurrence of migraine days, the number that were mild, moderate or severe, and the number that were associated with nausea or vomiting.

	No. of days with migraine	Severity			Migraine associated with:	
		Mild	Moderate	Severe	Nausea	Vomiting
Pre-menstruation (5 days before menstruation)	322	31	56	47	78	10
Post-menstruation (1 st day of bleeding +5 days)	889	85	136	138	210	32
All other times	1160	151	196	149	266	30
Total	2371	267	388	334	554	72

TABLE 2: THE TOTAL OCCURRENCE OF MIGRAINE DAYS, THE NUMBER THAT WERE SEVERE, MODERATE OR MILD, AND THE NUMBER ASSOCIATED WITH NAUSEA OR VOMITING

Figure 7 shows the number of attacks occurring on each day of the menstrual cycle, based on 126 women who had three consecutive cycles. The first day of bleeding was used as the reference point from which all other migraine days were compared. The frequency of migraines increased as menstruation was approached, with the highest frequency occurring on the first day of menstruation (day 1), and decreasing after. There was no association between migraine and the expected time of ovulation (day -14).

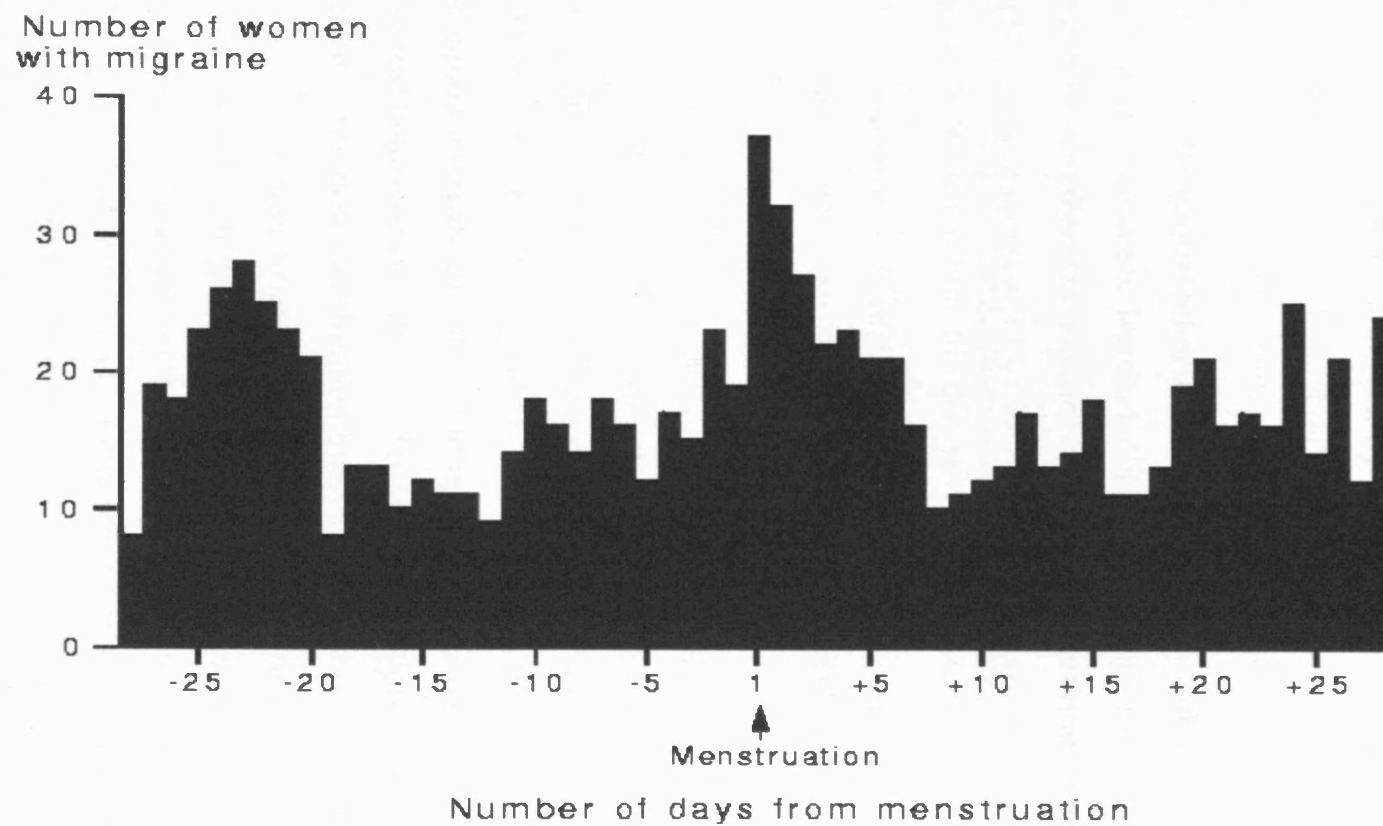


FIGURE 7: INCIDENCE OF MIGRAINE ON EACH DAY OF THE CYCLE IN 126 WOMEN WITH DATA FOR AT LEAST THREE CONSECUTIVE MENSTRUAL CYCLES

Table 3 shows the pooled relative risk of migraine during the pre-menstruation interval and post-menstruation interval compared to all other times of the cycle. In the five days preceding menstruation women were 25% more likely to have a migraine (relative risk 1.25). This increased to 71% in the two days before menstruation (relative risk 1.71). The chance of migraine was more than two-fold on the first day of menstruation and during the five days afterwards (relative risk 2.19). The risk was highest on the first day of menstruation and the following two days (relative risk 2.50). All the results were highly statistically significant.

Figure 8 shows the individual relative risk of migraine for each woman during the two days before menstruation. The horizontal line, relative risk of 1, indicates no difference in the occurrence of migraines during the menstrual cycle compared to at other times; 78% of women (117/150) had a relative risk that exceeded one (indicating increased risk during the pre-menstruation interval).

Figure 9 shows the individual relative risk of migraine for each woman during the first three days of menstruation; 76% of women (117/154) had a relative risk that exceeded one.

Table 4 shows the pooled relative risk of migraine according to severity. Severe attacks were more likely to occur during the pre-menstruation (relative risk 1.43) and post-menstruation intervals (relative risk 2.63) compared to all other times of the cycle. There was a trend from mild to severe in both the pre-menstruation and post-menstruation intervals, providing further weight that migraines tend to be more severe just before or after the first day of menstruation. Again, the risk was always highest in the post-menstruation interval than the pre-menstruation one. The risks were also greater when migraine attacks were restricted to the two days before or after (and including) the first day of menstruation. Women were more likely to have a severe migraine on the first day of menstruation or during the following two days (relative risk 3.41) compared to other times of her cycle. Again there was a trend from mild to severe. Results for the post-menstruation interval were highly statistically significant, and those results for severe migraines during the pre-menstruation interval were also generally significant.

	1 st day of menstruation ± 5 days				1 st day of menstruation ± 2 days			
	Relative risk	95% confidence interval	Statistical significance	Number of women*	Relative risk	95% confidence interval	Statistical significance	Number of women*
Pre-menstruation	1.25	1.10-1.42	p<0.001	150	1.71	1.45-2.01	p<0.0001	150
Post-menstruation	2.19	2.01-2.40	p<0.0001	155	2.50	2.24-2.77	p<0.0001	154
All other times [#]	1.00	-	-	-	1.00	-	-	-

Pre-menstruation is the 5 (or 2) days before menstruation; post-menstruation is the 1st day of bleeding and the subsequent 5 (or 2) days

* The number of women on whom the relative risk is based

[#] 'All other times' is defined as the days of the cycle other than day 1 of menstruation ± 5 days

TABLE 3: POOLED RELATIVE RISK OF MIGRAINE ON OR BETWEEN DAY 1±5 AND ON OR BETWEEN DAY 1±2

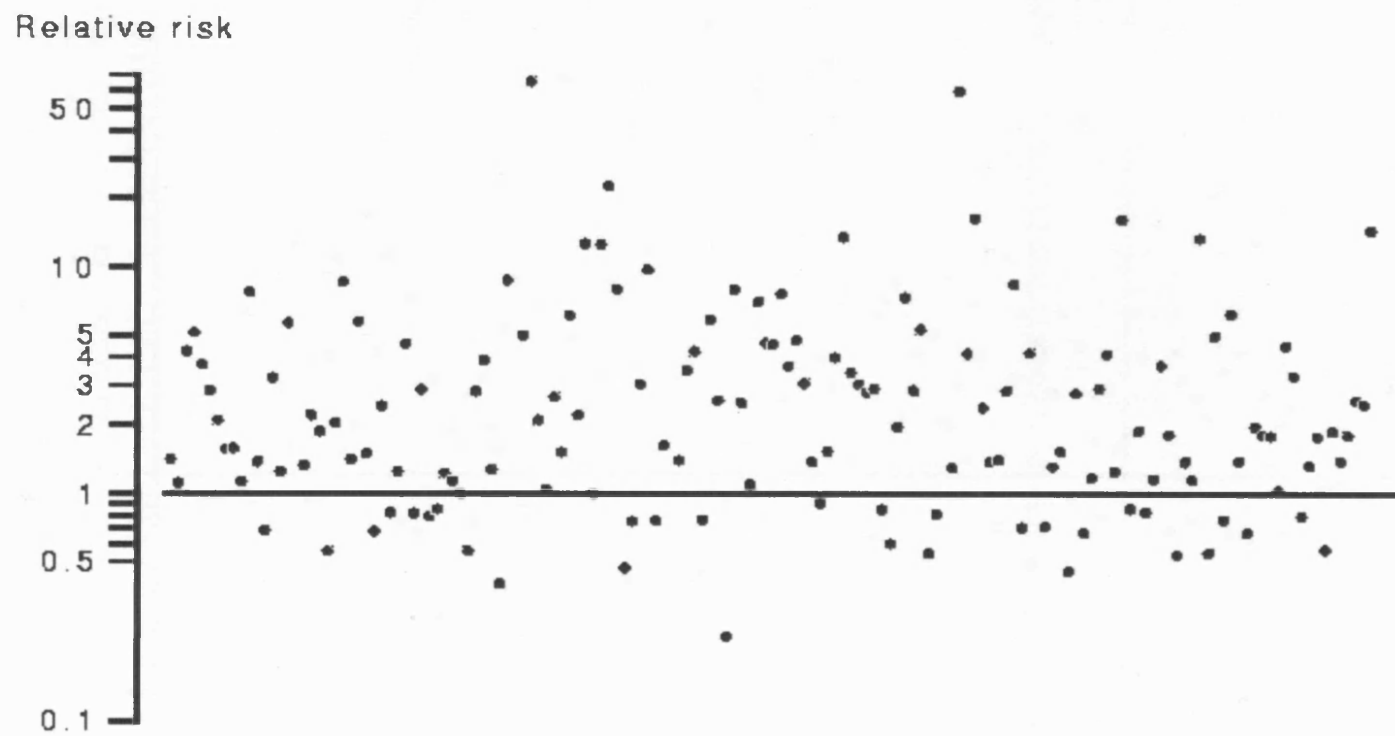


FIGURE 8: RELATIVE RISK OF MIGRAINE ON THE TWO DAYS BEFORE MENSTRUATION (N=154)

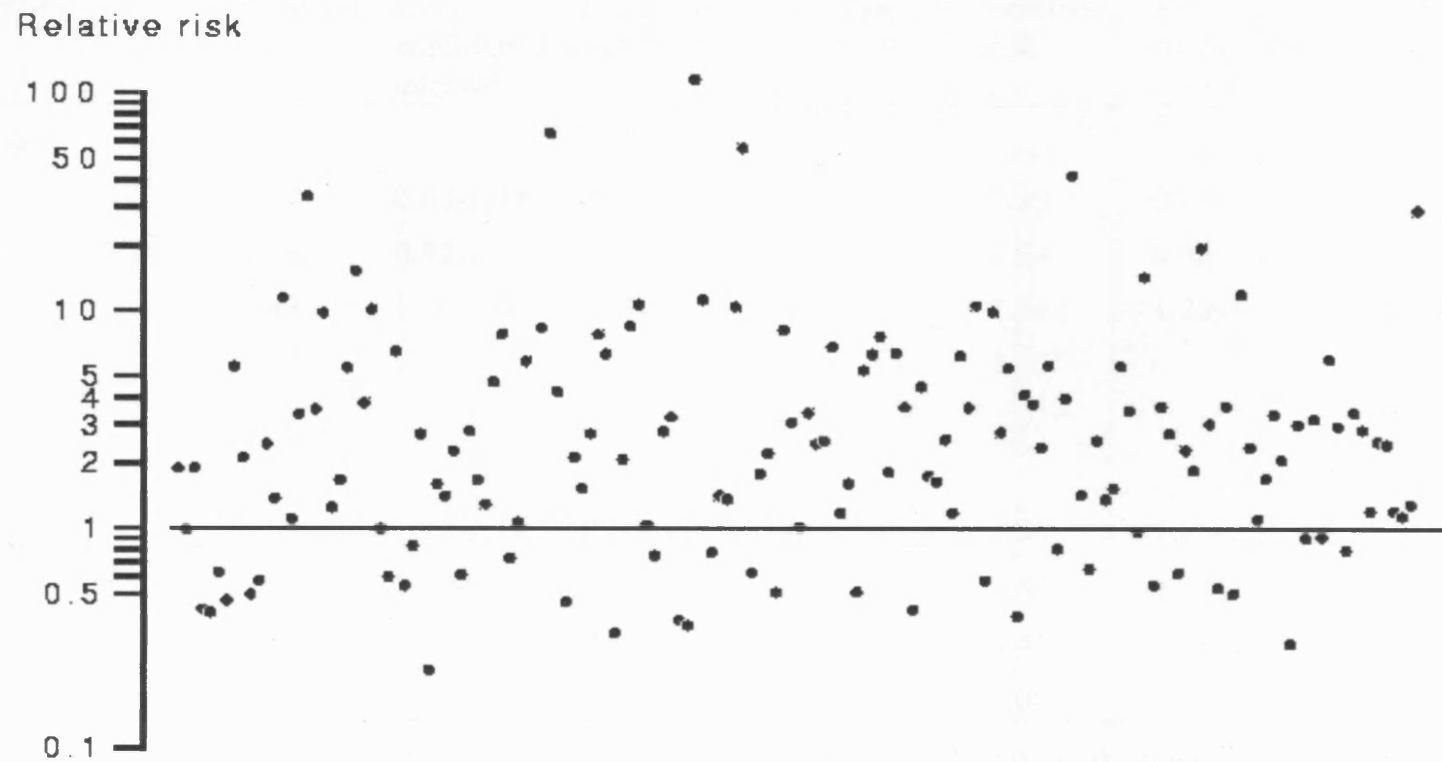


FIGURE 9: RELATIVE RISK OF MIGRAINE ON THE FIRST THREE DAYS OF MENSTRUATION (N=154)

Severity of migraine	1 st day of menstruation ± 5 days				1 st day of menstruation ± 2 days			
	Relative risk	95% confidence interval	Statistical significance	Number of women*	Relative risk	95% confidence interval	Statistical significance	Number of women*
<i>Pre-menstruation</i>								
Mild	0.97	0.63-1.43	<i>P</i> =0.95	43	1.39	0.78-2.28	<i>P</i> =0.27	42
Moderate	1.26	0.92-1.71	<i>P</i> =0.15	58	1.54	0.98-2.31	<i>P</i> =0.06	57
Severe	1.43	1.00-2.00	<i>P</i> =0.05	58	2.06	1.29-3.13	<i>P</i> =0.003	55
All other times	1.00	-	-	-	1.00 [#]	-	-	-
<i>Post-menstruation</i>								
Mild	1.62	1.22-2.12	<i>P</i> <0.001	48	1.54	1.05-2.19	<i>P</i> =0.03	42
Moderate	1.91	1.52-2.40	<i>P</i> <0.0001	66	1.89	1.41-2.51	<i>P</i> <0.0001	62
Severe	2.63	2.07-3.35	<i>P</i> <0.0001	59	3.41	2.58-4.47	<i>P</i> <0.0001	59
All other times [#]	1.00	-	-	-	1.00	-	-	-

Pre-menstruation is the 5 (or 2) days before menstruation; post-menstruation is the 1st day of bleeding and the subsequent 5 (or 2) days

* The number of women on whom the relative risk is based

[#] 'All other times' is defined as the days of the cycle other than day 1 of menstruation ± 5 days

TABLE 4: THE POOLED RELATIVE RISK OF MILD, MODERATE OR SEVERE MIGRAINE ON OR BETWEEN DAY 1±5 AND ON OR BETWEEN DAY 1±2

Table 5 shows the pooled relative risk of migraine associated with vomiting or nausea. Most women had migraine with nausea and migraine with vomiting was less common. Both types of migraine were more likely to occur close to menstruation, with highly statistically significant results observed for the post-menstruation interval (day 1 to day 6 of the cycle). Women were almost five times more likely to have a migraine associated with vomiting on or during days 1 to 3 of menstruation (relative risk 4.69).

Migraine associated with	1 st day of menstruation ± 5 days				1 st day of menstruation ± 2 days			
	Relative risk	95% confidence interval	Statistical significance	Number of women*	Relative risk	95% confidence interval	Statistical significance	Number of women*
<i>Vomiting:</i>								
Pre-menstruation	1.55	0.67-3.24	<i>P</i> =0.33	21	2.62	0.87-6.31	<i>P</i> =0.09	19
Post-menstruation	3.09	1.82-5.28	<i>P</i> <0.0001	24	4.69	2.62-8.31	<i>P</i> <0.0001	22
All other times	1.00	-	-	-	1.00 [#]	-	-	-
<i>Nausea:</i>								
Pre-menstruation	1.29	0.98-1.66	<i>P</i> =0.07	68	1.84	1.30-2.53	<i>P</i> <0.0001	66
Post-menstruation	2.18	1.81-2.63	<i>P</i> <0.0001	70	2.60	2.08-3.23	<i>P</i> <0.0001	69
All other times [#]	1.00	-	-	-	1.00	-	-	-

Pre-menstruation is the 5 (or 2) days before menstruation; post-menstruation is the 1st day of bleeding and the subsequent 5 (or 2) days

* The number of women on whom the relative risk is based

[#] 'All other times' is defined as the days of the cycle other than day 1 of menstruation ± 5 days

TABLE 5: THE POOLED RELATIVE RISK OF MIGRAINE ASSOCIATED WITH VOMITING OR NAUSEA ON OR BETWEEN DAY 1±5 AND ON OR BETWEEN DAY 1±2

DISCUSSION

The results of this observational study confirm a clear association between migraine and menstruation. Migraine was more likely to occur during a five-day window from two days before to two days after the first day of menstruation (days -2 to +3). The within-woman analysis showed that menstrual attacks also tended to be more severe and associated with nausea and vomiting at this time compared to attacks at other times of the cycle. The greatest effect of menstruation on migraine was during the first three days of the cycle.

These results are similar to previously published diary card studies showing that the peak time for migraine during the menstrual cycle is on or between two days before the start of menstruation and the first three days of bleeding. (Waters & O'Connor, 1971; Dalton, 1973; MacGregor *et al.*, 1990; Johannes *et al.*, 1995; Stewart *et al.*, 2000) Although the present study suggests that days +4 to +6 are also associated with increased risk of migraine, all migraine days were analysed whereas our 1990 study analysed only the first day of each migraine attack.

The strengths of the present study are the larger population, greater number of cycles, the confirmed diagnosis for each migraine attack, the accuracy of diary card data, and use of within-woman analysis.

We were unable to look at the association between migraine aura and menstruation. Women had been asked to complete the diaries as part of their clinical management rather than as participants in a clinical trial and it was noted that some women confused premonitory symptoms with aura. Although the true diagnosis of each attack could be established by direct questioning in the majority of cases, it was not always possible to be sure, particularly for women who had attacks with and without aura. Hence we considered that these data were potentially too unreliable for an analysis to be meaningful.

A potential weakness is that these data are from women attending a specialist headache clinic and cannot be extrapolated to the general population of women with migraine. However, these data are likely to represent women seeking medical help for migraine. Although a community based study would need to be undertaken to confirm this, previous studies in both community and clinic based

populations have shown similar results.(Waters & O'Connor, 1971; Dalton, 1973; MacGregor *et al.*, 1990; Johannes *et al.*, 1995; Stewart *et al.*, 2000; Couturier *et al.*, 2003)

SUMMARY OF FINDINGS

Migraine with menstruation is a significant problem for many women, who also report that menstrual attacks are of longer duration, more severe and less responsive to treatment compared to attacks at other times of the cycle. The present diary based observational study confirms that migraine is most likely to occur during the two days before menstruation and the first three days of bleeding, and that these attacks tend to be more severe than non-menstrual attacks. There was no association between migraine and ovulation.

1.2.2. ADDITIONAL EVIDENCE OF CLINICAL DIFFERENCES BETWEEN MENSTRUAL MIGRAINE AND NON-MENSTRUAL ATTACKS: A REVIEW

Based on incidence alone, menstrual migraine could be classified as a sub-category of migraine without aura with a predictable 'trigger'. On this basis, there would be no need for a specific definition.

In support of this, published reviews of data from large databases generated in clinical trials suggest that all seven of the commercially available triptans work as well for menstrual attacks as they do for attacks at other times of the month, which suggests there is no need for specific definition with respect to treatment strategies.(Solbach & Waymer, 1993; Facchinetti *et al.*, 1995; Gross *et al.*, 1995; Silberstein *et al.*, 1999; MacGregor & Keywood, 2000; Massiou *et al.*, 2000; Silberstein *et al.*, 2000; Silberstein *et al.*, 2002; Nett *et al.*, 2003; Loder *et al.*, 2004; Massiou *et al.*, 2005) However, the analyses were based on all attacks occurring during menstruation versus non-menstrual attacks and not based on attacks in women diagnosed with menstrual migraine.

In contrast, the results from Paper IV suggest that within individual women there are clinical differences between menstrual migraine and non-menstrual attacks. Other published research in women diagnosed with menstrual migraine according to the proposed definitions confirms that menstrual attacks of migraine are more severe than non-menstrual attacks but also that they last longer, are less responsive to treatment, and more prone to relapse.(Allais *et al.*, 2006; Couturier *et al.*, 2003; Dowson *et al.*, 2005; Granella *et al.*, 2004; Visser *et al.*, 1996)

In a prospective placebo-controlled study of 115 women, data from 53 women identified with menstrually-related migraine suggested that sumatriptan was less effective for menstrual attacks compared to non-menstrual attacks (Figure 10). (Gross et al., 1995; Dowson et al., 2005)

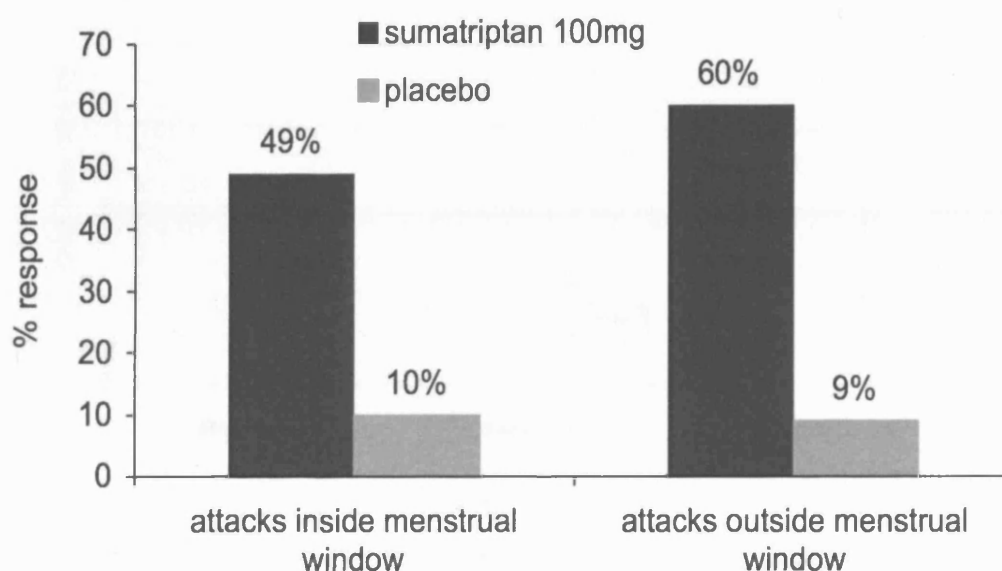


FIGURE 10: EFFICACY OF SUMATRIPTAN 100MG 4 HOURS POST TREATMENT ON ATTACKS INSIDE AND OUTSIDE THE MENSTRUAL WINDOW. (GROSS ET AL., 1995; DOWSON ET AL., 2005)

Granella *et al.* noted that pain-free at 2 hours following treatment was less likely to be achieved for menstrual versus non-menstrual attacks (Figure 11) and menstrual attacks lasted longer than non-menstrual attacks (Table 6). (Granella *et al.*, 2004)

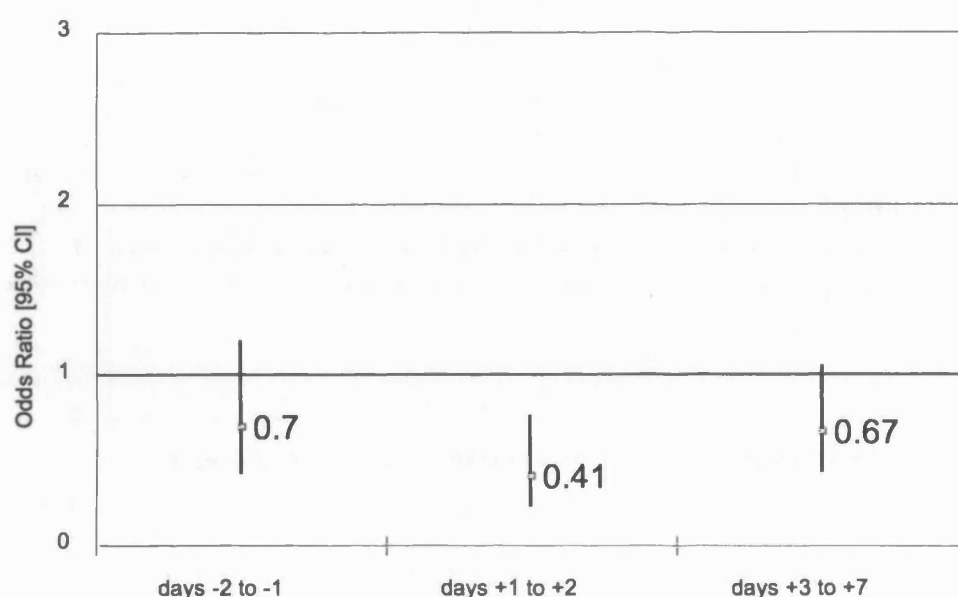


FIGURE 11: 2 HR PAIN-FREE: MENSTRUAL ATTACKS (DAYS -2 TO +3) VS. ALL OTHER TIMES OF THE CYCLE. (GRANELLA ET AL., 2004)

Day of menstrual cycle*	Attack duration (hrs) (mean \pm 95% CI)	Significance
Days -2 to -1	29.6 \pm 24.1	$P < 0.0001$
Days +1 to +2	33.7 \pm 24.8	$P < 0.0001$
Days +3 to +7	24.0 \pm 21.8	$P = 0.002$
All other times	16.3 \pm 15.1	

* relative to 1st day of bleeding (Day +1)

TABLE 6: DURATION OF MIGRAINE AT DIFFERENT STAGES OF THE MENSTRUAL CYCLE (GRANELLA ET AL., 2004)

Granella *et al.* also found that following treatment, menstrual attacks were more likely to relapse than non-menstrual attacks (Figure 12).(Granella *et al.*, 2004)

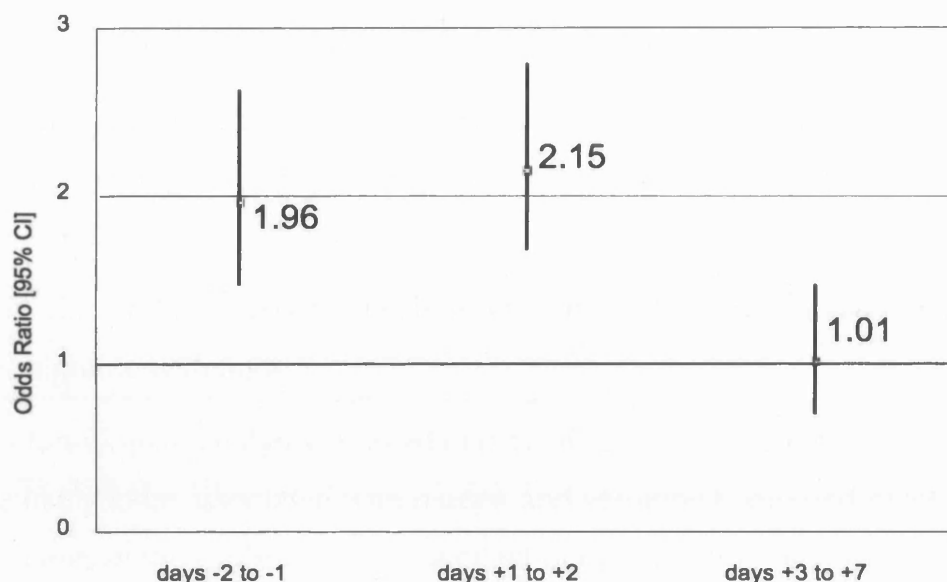


FIGURE 12: RELAPSE IN 24 HOURS: MENSTRUAL ATTACKS (DAYS -2 TO +3) VS. ALL OTHER TIMES OF THE CYCLE.(GRANELLA ET AL., 2004)

Similarly, Allais *et al.* reported that although acute triptan therapy for menstrual migraine provided effective pain-free relief at 2 hours in most women, a sustained pain-free state was achieved by fewer than 30% of patients.(Allais *et al.*, 2006)

SUMMARY OF FINDINGS

In contrast to post-hoc analyses of treatment trials, specific studies of women with menstrual migraine confirm the clinical impression that menstrual attacks are more severe, less responsive to symptomatic treatment and more prone to relapse compared to non-menstrual attacks. They also highlight the importance of the clinically relevant within-woman analyses of menstrual versus non-menstrual attacks, assessing intra-individual variation rather than inter-individual variation.

1.3. CONCLUSIONS FROM PART ONE

From all the data presented thus far, the following points can be concluded:

1. There is no agreed definition for menstrual migraine; clinical trials have used a variety of definitions, making it difficult to compare data.
2. In women with migraine, attacks are more likely to occur during a five-day window from two days before to two days after the first day of menstruation (days –2 to +3).
3. Migraine at this time of the cycle is typically *without* aura, even in women who have migraine *with* aura.
4. Within-woman analyses showed that menstrual attacks are more severe and more likely to be associated with nausea and vomiting compared to attacks at other times of the cycle; the greatest effect of menstruation on migraine is during the first three days of the cycle.
5. Other published data suggest that menstrual attacks are longer, less responsive to treatment, and more prone to relapse compared to non-menstrual attacks.
6. Subjective assessment overestimates the association between migraine and menstruation and accurate diary data is mandatory to confirm the diagnosis.

It could be argued that menstrual migraine does not require a discrete definition and should be considered as migraine without aura. Menstruation is just an additional trigger to others such as missed or delayed meals, dehydration, lack of sleep, etc.

However, evidence supports the hypothesis that menstrual migraine is a distinct clinical entity since menstrual attacks are clinically different from attacks at other times of the cycle.

To ensure the association with menstruation is not a chance occurrence, it is necessary to ensure that the association is consistent over several cycles. As a minimum, it seems reasonable that the association could be confirmed if menstrual attacks occur in at least two out of three menstrual cycles.

On the basis of the above findings, the following definitions for pure menstrual migraine and menstrually-related migraine were proposed to encompass the clinical entity of menstrual migraine:

Pure menstrual migraine

Attacks of migraine without aura occurring exclusively on or between days -2 to +3 of menstruation in at least two out of three menstrual cycles and at no other times of the cycle

Menstrually-related migraine

Attacks of migraine without aura occurring on or between days -2 to +3 of menstruation in at least two out of three menstrual cycles with additional attacks of migraine with or without aura at other times of the cycle

These definitions were submitted to Headache Classification Committee of the International Headache Society for consideration and are included in the second edition of the International Classification of Headache Disorders. (Headache Classification Subcommittee of the International Headache Society (IHS), 2004) At present, they are in the appendix rather than part of the main classification, in consideration of the need for additional confirmatory research and epidemiological evidence. An agreed definition is essential to ensure that comparable populations are studied, so that we can elucidate the pathophysiology and improve management. Research will also enable further refinement of the definitions.

1.4. AN INTERNATIONAL CLASSIFICATION FOR MENSTRUAL MIGRAINE

A1.1.1 Pure menstrual migraine without aura

Diagnostic criteria:

- A. Attacks, in a menstruating woman, fulfilling criteria for migraine without aura
- B. Attacks occur exclusively on day 1 \pm 2 (i.e., days -2 to +3)¹ of menstruation² in at least two out of three menstrual cycles and at no other times of the cycle

A1.1.2 Menstrually-related migraine without aura

Diagnostic criteria:

- A. Attacks, in a menstruating woman, fulfilling criteria for migraine without aura
- B. Attacks occur on day 1 \pm 2 (i.e., days -2 to +3)¹ of menstruation² in at least two out of three menstrual cycles and additionally at other times of the cycle

Notes:

1. The first day of menstruation is day 1 and the preceding day is day -1; there is no day 0
2. For the purposes of this classification, menstruation is considered to be endometrial bleeding resulting from either the normal menstrual cycle or from the withdrawal of exogenous progestogens, as in the case of combined oral contraceptives and cyclical hormone replacement therapy

PART 2:
MENSTRUAL MIGRAINE IS ASSOCIATED WITH OESTROGEN
'WITHDRAWAL'

2.1 MIGRAINE AND OESTROGEN 'WITHDRAWAL' DURING THE NATURAL MENSTRUAL CYCLE

2.1.1. EVIDENCE FOR THE ASSOCIATION BETWEEN MIGRAINE AND OESTROGEN 'WITHDRAWAL' DURING THE NATURAL MENSTRUAL CYCLE: A REVIEW

The inconsistency of definitions for 'menstrual' migraine has made it impossible to establish the true significance of the results of biochemical and hormonal studies previously published. The steroid hormones involved in the menstrual cycle, particularly oestrogen and progesterone, are the obvious targets for research. Studies comparing levels of these hormones in women with 'menstrual' migraine versus controls have not identified any convincing differences. (Epstein *et al.*, 1975) However, such findings do not exclude sensitivity to normal endocrine changes of the ovarian cycle as a 'trigger' for attacks.

THE MENSTRUAL CYCLE

The average menstrual cycle lasts 28 days - counted from the first day of one menstrual period to the start of the next - but this can vary considerably in individuals.

The cycle can be divided into three stages: the follicular phase, ovulation, and the luteal phase (Figure 13).

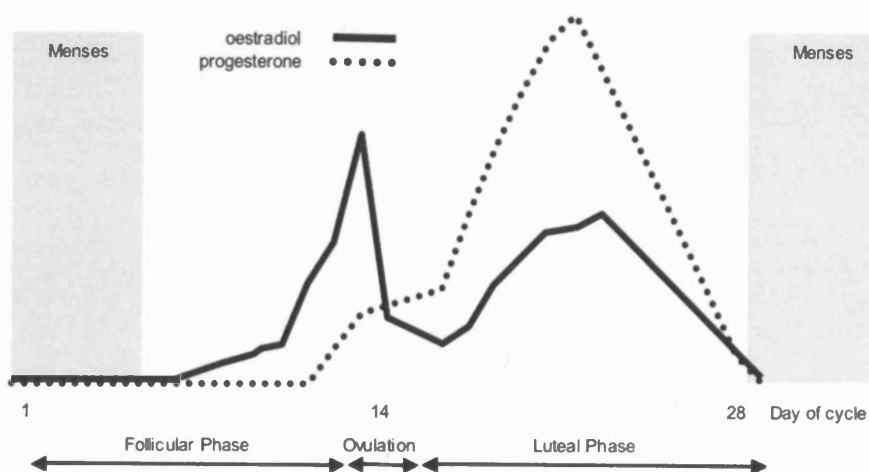


FIGURE 13: THE THREE STAGES OF THE MENSTRUAL CYCLE

The follicular phase is the first part of the cycle. At the start of each cycle, hormones from the hypothalamus stimulate the pituitary gland to secrete follicle stimulating hormone (FSH)① (Figure 14). This stimulates the ovaries and five to twenty follicles rapidly start to grow. By about day six of the cycle, a single follicle in one of the ovaries grows faster, while the others regress.

The developing follicles produce oestrogen②. Rising oestrogen levels feedback to the pituitary gland, to reduce the secretion of FSH and trigger luteinizing hormone (LH)③. Ovulation takes place as a surge of LH causes the follicle to rupture, releasing the mature egg④.

The luteal phase is the time between ovulation and menstruation. It consistently lasts close to 14 days.(Speroff & Fritz, 2004) Under the control of LH, the empty follicle transforms into the corpus luteum, which produces progesterone and a second rise of oestrogen⑤. Therefore progesterone is only present in the luteal phase of ovulatory cycles. High hormone levels cause the production of follicle stimulating hormone and luteinizing hormone to fall⑥. If there is no pregnancy, the corpus luteum rapidly degenerates, nine to eleven days after ovulation and levels of oestrogen and progesterone fall. The fall in progesterone causes the menstrual bleed⑦.

Given the increased incidence of migraine at this time of the cycle, the luteal fall in oestrogen and progesterone merits further examination.

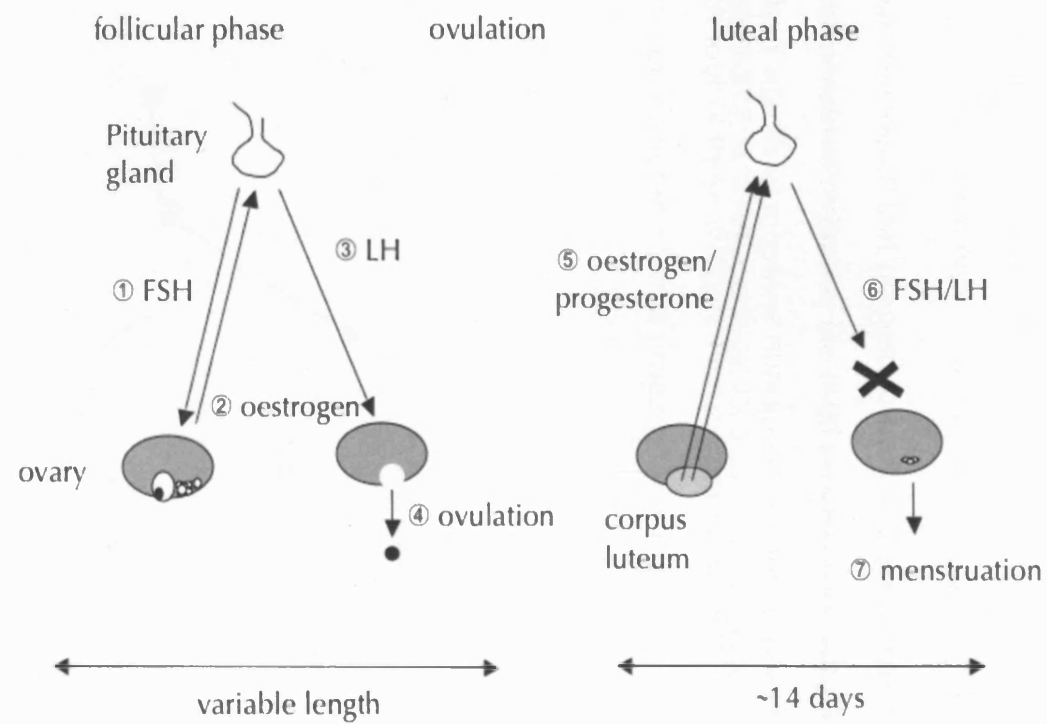


FIGURE 14: THE PITUITARY-OVARIAN AXIS: FOLLICULAR, OVULATORY, AND LUTEAL PHASES

OESTROGEN AND PROGESTERONE IN MENSTRUAL MIGRAINE

Progesterone

Insufficient progesterone in the luteal phase was first considered to be responsible for the occurrence of menstrual migraine. (Gray, 1941; Singh & Singh, 1947)

A more recent study of 14 volunteers, 11 of whom had menstrual migraine, suggested that luteal progesterone levels were significantly associated with headache activity. (Beckham *et al.*, 1992) However, the number of patients was small and samples were taken only at four points during a single menstrual cycle.

Somerville also considered that progesterone 'withdrawal' was a likely mechanism for menstrual migraine. He used progesterone supplements to treat six women who had attacks of migraine during the late luteal phase. Menstruation was delayed in four of these women. Five experienced migraine at their customary time, unrelated to plasma progesterone levels (Figure 15). (Somerville, 1971, 1972a)

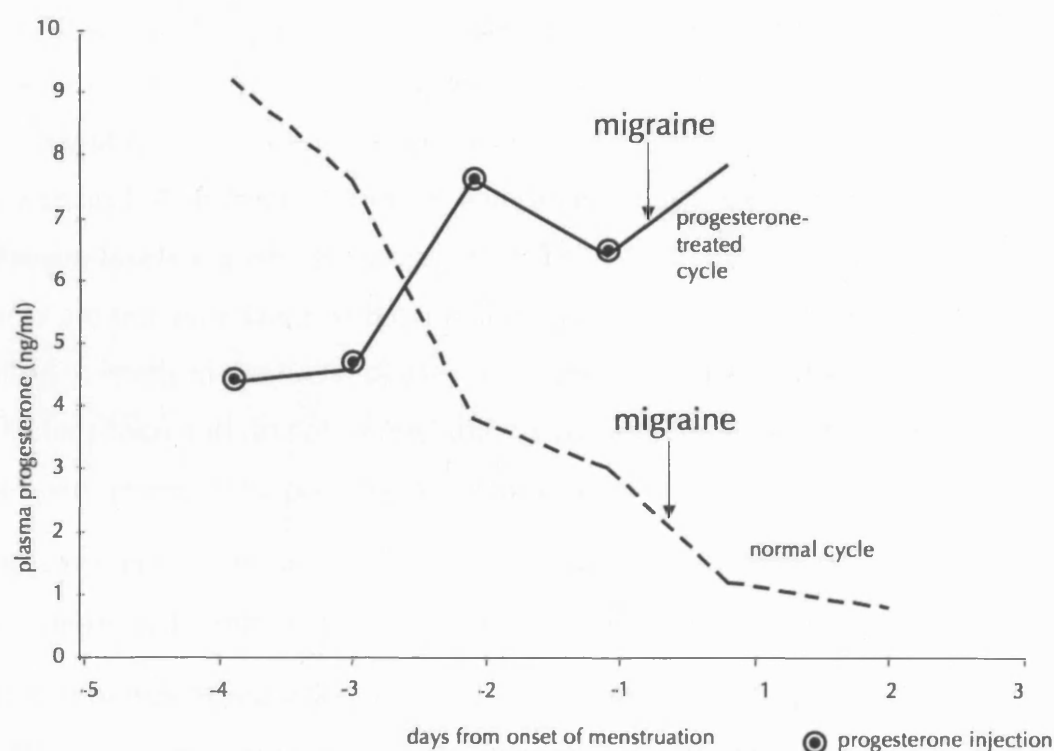


FIGURE 15: THE EFFECT OF TREATMENT WITH PROGESTERONE ON MENSTRUAL MIGRAINE. (SOMERVILLE, 1971, 1972A)

Somerville also studied the effects of exogenous progesterone in women in whom high serum levels of exogenous oestrogen had inhibited ovulation. Neither the administration of progesterone nor its subsequent 'withdrawal' resulted in migraine.

In contrast, continuous progestogen treatments at doses sufficient to suppress the endogenous menstrual cycle, have been effective in preventing menstrual migraine.(Bradley *et al.*, 1968; Somerville & Carey, 1970)

Oestrogen

The evidence is more in favour of menstrual attacks being associated, at least in some women, with falling levels or 'withdrawal' of oestrogen. Somerville went on to examine the effects of oestrogen on menstrual migraine, studying 14 women each of whom had a predictable attack of migraine every month, always confined to the premenstrual or menstrual phases.(Somerville, 1972b, 1975b, a) Each subject had confirmed menstrual migraine for at least six successive cycles immediately prior to the study. A review of his data shows that all the subjects had attacks occurring on or between day 1 ± 3 of the menstrual cycle. On the basis of his observations, Somerville suggested that migraine could be triggered by the sudden withdrawal of oestrogen following high oestrogen levels. He also noted that a period of oestrogen 'priming' with several days of exposure to high oestrogen levels is a necessary precursor. This would explain why migraine attacks are not associated with ovulation. In contrast to sustained higher oestrogen levels in the luteal phase, oestrogen levels are relatively low in the follicular phase and do not 'prime' the system sufficiently for the drop in oestrogen immediately post ovulation to have an effect.

If the oestrogen 'withdrawal' hypothesis is correct, stabilising oestrogen fluctuations by maintaining high, stable levels, should prevent migraine.

In favour of this, Somerville showed that migraine could be postponed by maintaining high plasma oestradiol levels with an intramuscular injection of long-acting oestradiol valerate in oil; migraine subsequently occurred when the plasma oestradiol fell (Figure 16).

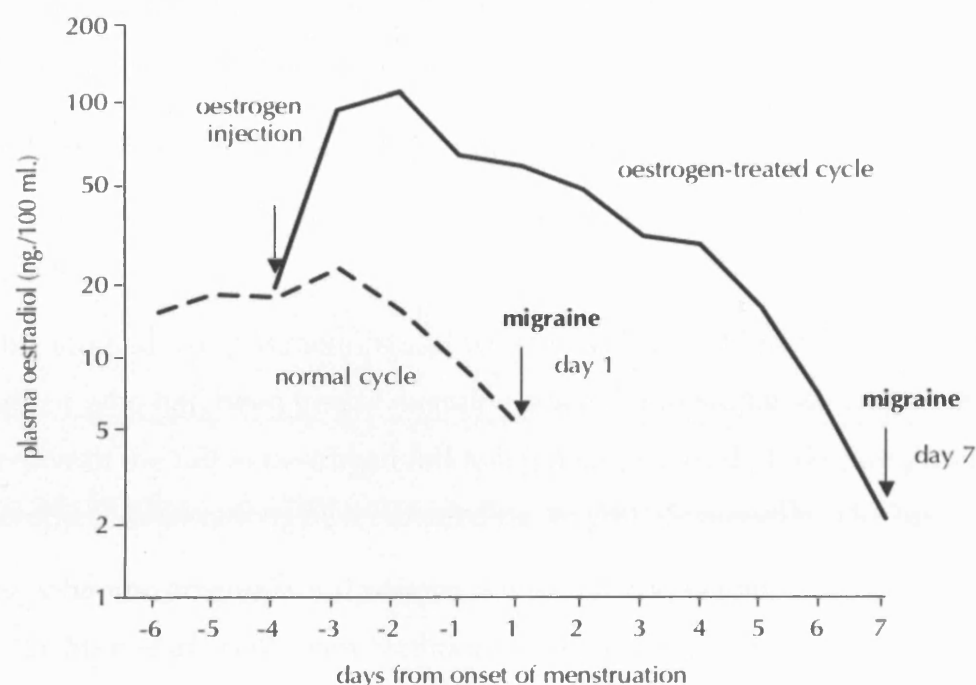


FIGURE 16: PLASMA OESTRADIOL CONCENTRATIONS DURING THE PREMENSTRUAL AND MENSTRUAL PHASES OF NORMAL AND OESTRADIOL-TREATED CYCLES IN ONE WOMAN WITH REGULAR MENSTRUAL MIGRAINE.(SOMERVILLE, 1972B)

Somerville also tried a short-acting oestrogen, which did not produce the same results, confirming his suggestion that prolonged oestrogen exposure is necessary for 'withdrawal' to trigger migraine. Further attempts to control oestrogen fluctuations with oral oestradiol valerate and conjugated equine oestrogens, failed.(Somerville, 1975b) These formulations did not significantly affect plasma oestradiol levels, which is likely to account for their failure to prevent migraine.

Several other studies support Somerville's oestrogen withdrawal theory. Epstein *et al.*, noted that the extent of decline from peak to trough oestrogen was greater in all 14 women with migraine in their study compared to 8 women in the control group who did not have migraine.(Epstein *et al.*, 1975) They concluded that variation in hormonal activity might be a potentially relevant factor in all women

with migraine; factors additional to the hormonal environment could account for the development of 'menstrual' attacks. Lichten *et al.*, studied 28 postmenopausal women challenged with oestrogen confirming that a drop in serum oestrogen could precipitate migraine attacks and that a period of oestrogen priming was a necessary prerequisite.(Lichten *et al.*, 1996)

Oestrogen 'withdrawal' in the absence of progesterone

Several studies suggest that oestrogen 'withdrawal' migraine is independent of progesterone.

Somerville studied two postmenopausal women with a past history of menstrual migraine but who had been free of migraine since the menopause. Migraine was associated with the fall in oestrogen following depot oestradiol, despite plasma progesterone concentrations never exceeding 1ng/mL.(Somerville, 1972a)

Similarly, when postmenopausal women were challenged with oestrogen in the study by Lichten *et al.*, oestrogen 'withdrawal' migraine occurred in the absence of progesterone and a period of oestrogen priming was a necessary prerequisite.(Lichten *et al.*, 1996)

Additional clinical evidence for oestrogen 'withdrawal'

Women taking the combined oral contraceptive pill can experience migraine attacks during the pill-free week, when ethinyloestradiol falls after 21 days of pill-taking.(Whitty *et al.*, 1966) In women using hormone replacement therapy, migraine attacks occurred during the week free from oestrogen in the old regime of 21 days on treatment, 7 days off.(Kudrow, 1975) In a placebo-controlled double-blind crossover study of hysterectomised women with bilateral oophorectomies, increased frequency of headache was reported following courses of oestrogen.(Dennerstein *et al.*, 1978) Migraine attacks also occur directly postpartum, a time when oestrogen levels plummet.(Stein, 1981) These findings provide further evidence for oestrogen 'withdrawal' migraine and together with the studies in postmenopausal women, suggest that ovulation is not a necessary precursor.

However, these studies were undertaken on small numbers of women over few cycles. Larger studies were warranted to confirm these findings.

2.1.2. PAPER V: INCIDENCE OF MIGRAINE RELATIVE TO MENSTRUAL CYCLE PHASES OF RISING AND FALLING OESTROGEN

This longitudinal study was undertaken to assess the association between migraine and urinary oestrogen levels on each day of the menstrual cycle. All women were diagnosed with menstrual migraine and were not using exogenous hormones.

OBJECTIVES

The objectives of the study were to assess the association between migraine and urinary hormone levels, with particular reference to rising and falling phases of oestrogen, and to test the hypothesis that oestrogen 'withdrawal' triggers migraine in the late luteal phase of the menstrual cycle.

METHODS

Participants were selected from women attending the City of London Migraine Clinic who had been keeping diary card records of migraine and menstruation. They were eligible for inclusion into the study if they had pure menstrual migraine or menstrually-related migraine, and had regular menstrual cycles (21-35 days).

Eligibility was based on prospective diary information recorded for at least three months, to confirm menstrual migraine or menstrually-related migraine. Women with additional non-migraine headaches could participate provided that they could distinguish these headaches from migraine. Non-hormonal migraine prophylaxis could be continued provided that the dose remained stable during the study period.

Women were not eligible if: headaches (including migraine headaches) occurred more often than three days a week on average, i.e. more than 12 days per month; analgesics or acute headache medications were used regularly on more than three days a week; they were pregnant, or intended to become pregnant during the study period (women used non-hormonal contraception), or were breastfeeding; had evidence of impaired liver or kidney function; had evidence of

polycystic ovarian syndrome (unless they had regular menstrual periods); used hormonal contraception, hormone replacement therapy or other hormonal treatment within six months prior to the start of the study or at any time during the study period; used treatments that might affect the menstrual cycle such as a diet high in soy, or use of soy/isoflavane supplements; be taking tetracycline (which affects LH assay) or had any medical or psychiatric condition that might have been affected adversely by use of oestrogen supplements or which would preclude participation in the study.

All women gave informed consent and were reviewed monthly, either by telephone or by a clinic visit. All study documents were approved by an independent Ethics Committee.

DEFINITIONS

Pure menstrual migraine was defined as migraine attacks on or between day 1 of menstruation \pm 2 days (i.e. on or between days -2 to $+3$ of the cycle assuming day 1 is the first day of menstruation and that there is no day 0) in at least two out of three cycles, with no migraine at other times of the cycle.

Menstrually-related migraine was defined as up to four attacks of migraine per month of which one must occur on or between day 1 of menstruation \pm 2 days in at least two out of three cycles.

INTERVENTIONS

Diary Cards

Women were provided with a diary card for each month on which the following information about each migraine attack was recorded, as it occurred: date and time of onset of symptoms; peak severity (mild, moderate or severe); duration of attack (to nearest day); associated symptoms (nausea, photophobia and phonophobia); aura, if present; medication (name, dose, time taken). If there was doubt about the nature of any headache episode, the patient was contacted for clarification.

Urine Samples

Daily early morning urine (EMU) samples were collected on each morning of the study, in universal vials containing sodium azide (0.1%) as a preservative. During the collection period, women sent their samples to the laboratory weekly where specimens were aliquoted and stored at 4°C until analyses were carried out.

Hormonal Analyses

Urine samples were analysed for oestrone-3-glucuronide (E₁G) and pregnanediol 3-glucuronide (PdG), urinary metabolites of oestradiol and progesterone, together with luteinizing hormone (LH), and follicle-stimulating hormone (FSH) using an AutoDelfia® (Perkin Elmer Life Sciences, Cambridge, UK). LH was used to confirm ovulation, while FSH allowed classification of menopause status.(Miro *et al.*, 2004c) A standard curve comprising six standards (in triplicate) and three quality control samples were run for each assay. Twenty-five replicates of each standard were run as samples and the concentration of each was determined using Multicalc®. The lowest detectable concentration of the assays was determined to be 0.09 IU/L (LH); 0.17IU/L (FSH); 0.1ng/ml (E₁G); and 0.07µg/ml (PdG). Samples were analysed in duplicate.

Urinary E₁G reflects the changes of plasma oestradiol.(Catalan *et al.*, 1989) Early morning urine samples were convenient and the excretion pattern of E₁G in EMU is similar to the pattern obtained for 24-hour urine samples.(World Health Organization, 1983) Because of this, concentrations can be expressed in mass/volume instead of mass steroid/creatinine ratio.(Adlercreutz *et al.*, 1982) Further, creatinine adjustment is not essential for the study of menstrual cycle urinary hormones based on daily sampling.(Miro *et al.*, 2004a) Data were shifted back one day for comparison with the diary card data, since morning collection of E₁G and PdG reflect serum hormone levels 12-24 hours earlier.(Munro *et al.*, 1991)

STATISTICAL METHODS

Rather than dividing the menstrual cycles into the standard follicular, ovulatory and luteal phases, each menstrual cycle was divided into phases of rising and falling oestrogen. Hence there were two phases of rising oestrogen (the first

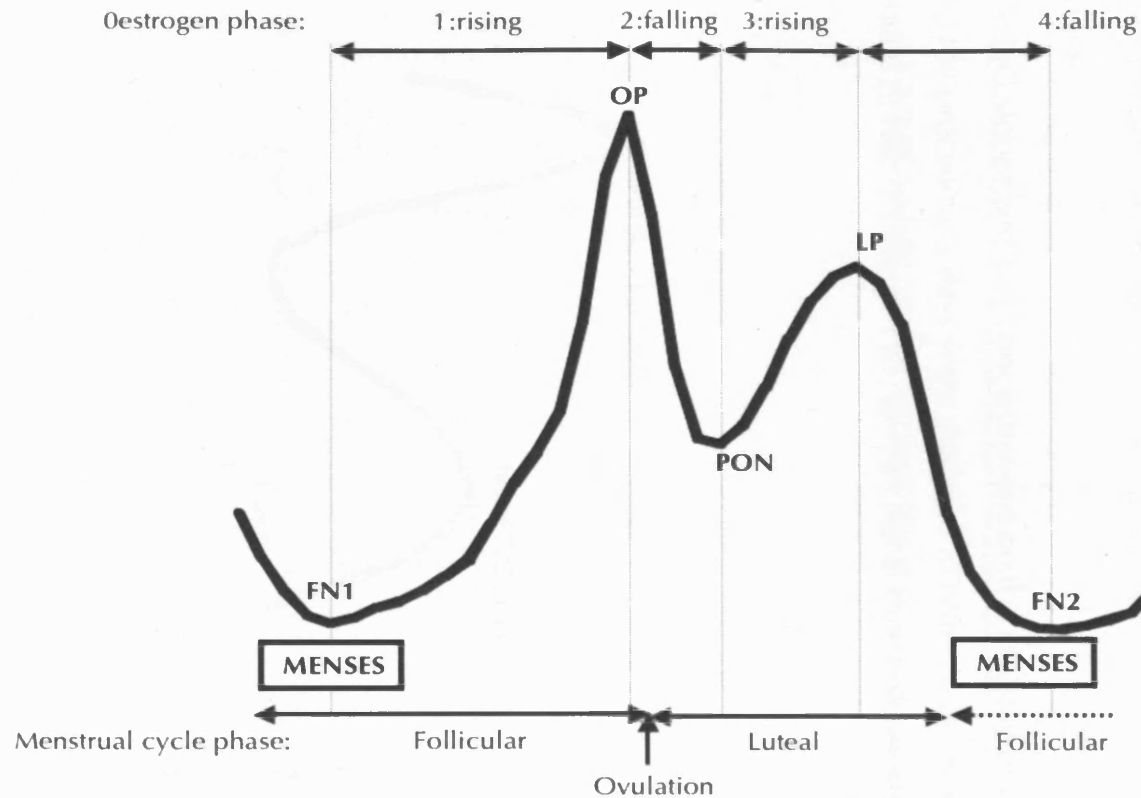
during the early follicular phase; the second during the early luteal phase) and two phases of oestrogen 'withdrawal' (the first occurring during early luteal phase; the second during late luteal phase) identified as follows (Figure 17):

Phase 1: follicular phase rising oestrogen - from the follicular E₁G nadir to the ovulatory peak.

Phase 2: post-ovulatory falling oestrogen - from the ovulatory peak to the post-ovulatory nadir.

Phase 3: luteal phase rising oestrogen - from the post-ovulatory nadir to the luteal E₁G peak.

Phase 4: late luteal/early follicular falling oestrogen - from the luteal E₁G peak to the E₁G nadir of the next cycle.



Phase 1: follicular phase rising oestrogen - from follicular E₁G nadir (FN1) to ovulatory peak (OP); Phase 2: post-ovulatory falling oestrogen - from ovulatory peak (OP) to post-ovulatory nadir (PON); Phase 3: luteal phase rising oestrogen - from the post-ovulatory nadir (PON) to the luteal E₁G peak (LP); Phase 4: late luteal/early follicular falling oestrogen - from the luteal E₁G peak to the E₁G nadir of the next cycle (FN2)

FN1= follicular nadir of cycle 1; OP = ovulatory peak; PON = post-ovulatory nadir; LP = luteal peak; FN2 = follicular nadir of cycle 2

FIGURE 17: EXAMPLE OF RISING AND FALLING PHASES OF OESTRONE-3-GLUCURONIDE (E₁G) ACROSS ONE MENSTRUAL CYCLE

A migraine attack was counted as consecutive days of migraine. An interval of 48 hours freedom from symptoms defined discrete attacks. (International Headache Society Clinical Trials Subcommittee, 2000) A migraine day was each day on which a woman reported migraine.

For each cycle the total number of migraine attacks in each phase was determined. The expected number, assuming random occurrence, was calculated as the total number times the ratio of the phase length to the cycle length. The expected and observed numbers in each phase were then cumulated over all cycles.

The E_1G slopes and E_1G concentrations on the first day of each migraine attack and the preceding 5 days were analysed in order to assess whether migraine was related to rate of change E_1G , or if a critical threshold existed (Figure 18).

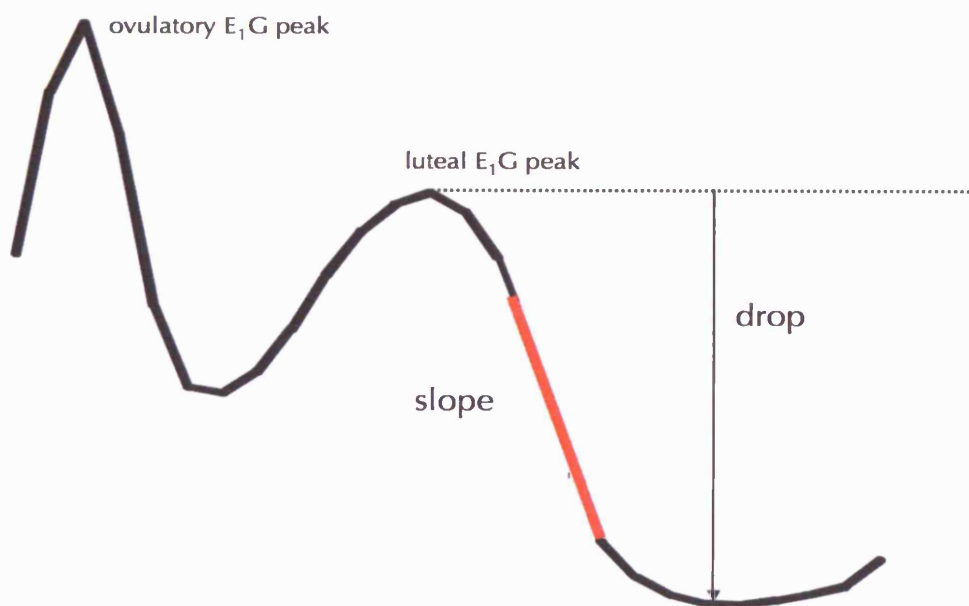


FIGURE 18: SCHEMATIC TO SHOW HOW SLOPE AND DROP OF E_1G WERE MEASURED

For each woman, the risk of migraine during the pre- or post-menstruation period (day 1 ± 2) was compared with the risk of migraine at all other times of her cycle, and the relative risk calculated. The relative risks were then combined across all women using a method that allows for small occurrences and provides a p-value and 95% confidence interval.(MacGregor & Hackshaw, 2004)

RESULTS

Forty women with diary evidence of pure menstrual migraine or menstrually-related migraine in two out of three cycles, and who fulfilled the eligibility criteria, were recruited into the study. Two women were excluded from the data analysis: one experienced cycles longer than the maximum 42 day cycle length acceptable for inclusion in the study; another with pre-study diaries confirming migraine with menstruation did not experience menstrual attacks during the study period. Therefore the final analyses were undertaken on data from 38 women.

Patient Characteristics

The mean age of the women was 43 (range 29-50) years; 87% were over age 35 and 29% were over age 45. All women had migraine without aura; one woman also had attacks of migraine with aura but all her menstrual attacks were without aura. Most women had menstrually-related migraine; one woman had pure menstrual migraine.

Nine women (24%) used analgesics alone for symptomatic treatment: 21(55%) used analgesics and triptans, 6 (16%) used triptans alone, 2 (5%) used ergots. Nine women (24%) were taking daily non-hormonal prophylaxis: 3 amitriptyline; 3 beta-blockers, 1 pizotifen, 1 amitriptyline and atenolol, 1 fluoxetine and pizotifen. There were no differences in demographic data or migraine frequency between these women and women who were not using prophylaxis.

Characteristics of migraine attacks

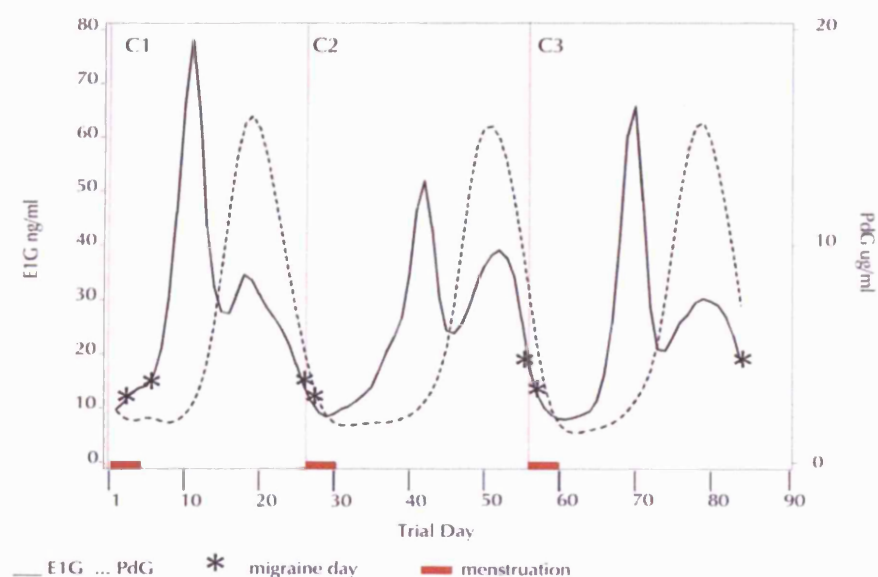
There was a total of 476 migraine days during the study. Women experienced a mean of 1.86 migraine attacks per cycle and 3.98 migraine days per cycle.

Migraine was more likely to occur on day 1 ± 2 compared to all other times of the cycle (RR 1.45 [95%CI 1.17-1.79], $p < 0.001$). Further, these attacks were more likely to be moderate or severe (RR 1.35 [95%CI 1.03-1.74], $P = 0.03$) and

associated with nausea (RR 1.46 [95%CI 1.04-2.00], $P=0.03$) or vomiting (RR 2.56 [95%CI 1.05-5.78], $P=0.04$).

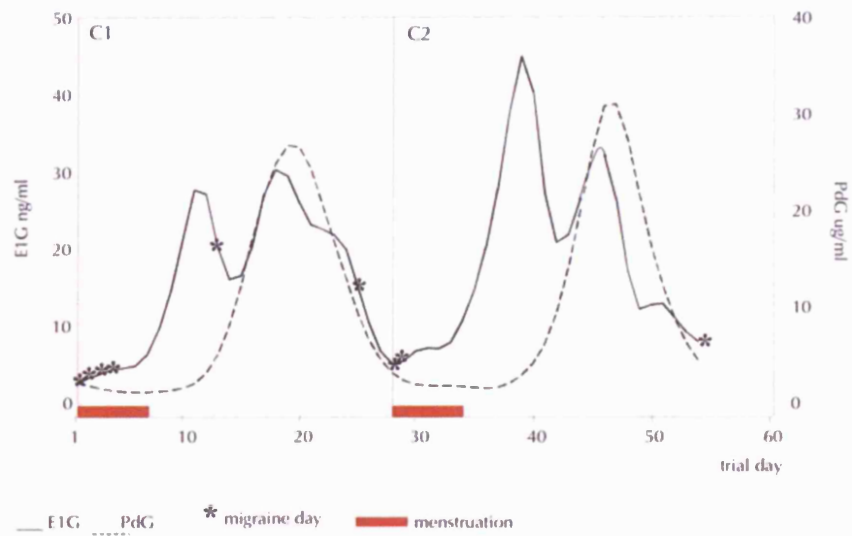
Association between migraine and urinary hormone levels

Cycle day, E₁G levels, PdG levels, menstruation, and migraine days as collected for all women are represented in Figures 19 and 20. These show information from two women: one with pure menstrual migraine and one with menstrually-related migraine. This highlights the intra-individual variation in hormone levels and timing of migraine over consecutive cycles.



E₁G = oestrone-3-glucuronide; PdG = pregnanediol-3-glucuronide

FIGURE 19: URINARY HORMONE LEVELS, MENSTRUATION AND DAYS OF MIGRAINE OVER 3 CYCLES IN ONE VOLUNTEER WITH PURE MENSTRUAL MIGRAINE.



E1G = oestrone-3-glucuronide; PdG = pregnanediol-3-glucuronide

FIGURE 20: URINARY HORMONE LEVELS, MENSTRUATION AND DAYS OF MIGRAINE OVER 2 CYCLES IN ONE VOLUNTEER WITH MENSTRUALLY-RELATED MIGRAINE

Migraine incidence was inversely related to rising and falling phases of oestrogen (Table 7). The differences between phases were highly significant ($p < 0.0013$). Phase 4 (late luteal/early follicular falling oestrogen) had the highest incidence of migraine compared to the expected incidence. In contrast, phases of rising oestrogen (phase 1 and phase 3) had the lowest incidence of migraine compared to the expected incidence. There was no significant difference between expected and observed number of migraine attacks at the time of post-ovulatory falling oestrogen (phase 2).

	Observed no. of migraine attacks	Expected no. of migraine attacks	χ^2
Phase 1: follicular phase rising oestrogen	52	64.4	2.39
Phase 2: post-ovulatory falling oestrogen	40	42.4	0.13
Phase 3: luteal phase rising oestrogen	23	33.5	3.29
Phase 4: late luteal/early follicular falling oestrogen	90	64.7	9.87
TOTAL			15.68
Overall $p < 0.0013$ for observed versus expected			

TABLE 7: OBSERVED AND EXPECTED FREQUENCY OF MIGRAINE DURING DIFFERENT PHASES OF THE MENSTRUAL CYCLE

Figure 21 shows the pooled incidence of migraine correlated with urinary E_1G and PdG on each cycle day of 120 cycles from 38 women. The incidence of migraine began to rise three days before the first full day of bleeding (day -3), associated with falling levels of E_1G . Peak incidence was on the first full day of bleeding (day +1) and the preceding day (day -1). The median cycle day of the E_1G nadir was day +2. As E_1G began to rise, the incidence of migraine declined. The mean peak luteal phase E_1G was 32.8ng/mL.

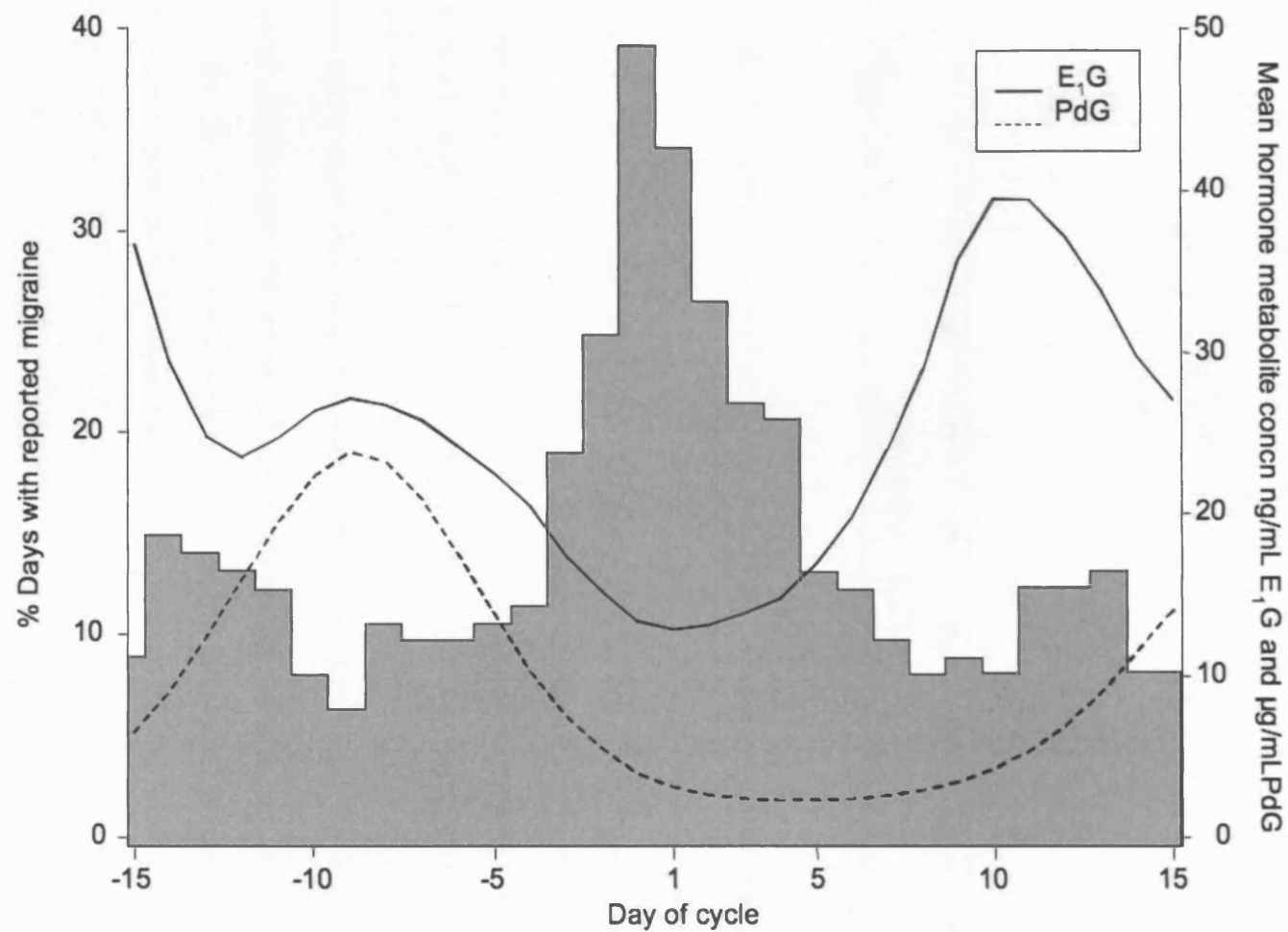


FIGURE 21: INCIDENCE OF MIGRAINE, URINARY OESTRONE-3-GLUCURONIDE (E₁G) AND PREGNANEDIOL-3-GLUCURONIDE (PdG) LEVELS ON EACH DAY OF THE MENSTRUAL CYCLE IN 120 CYCLES FROM 38 WOMEN

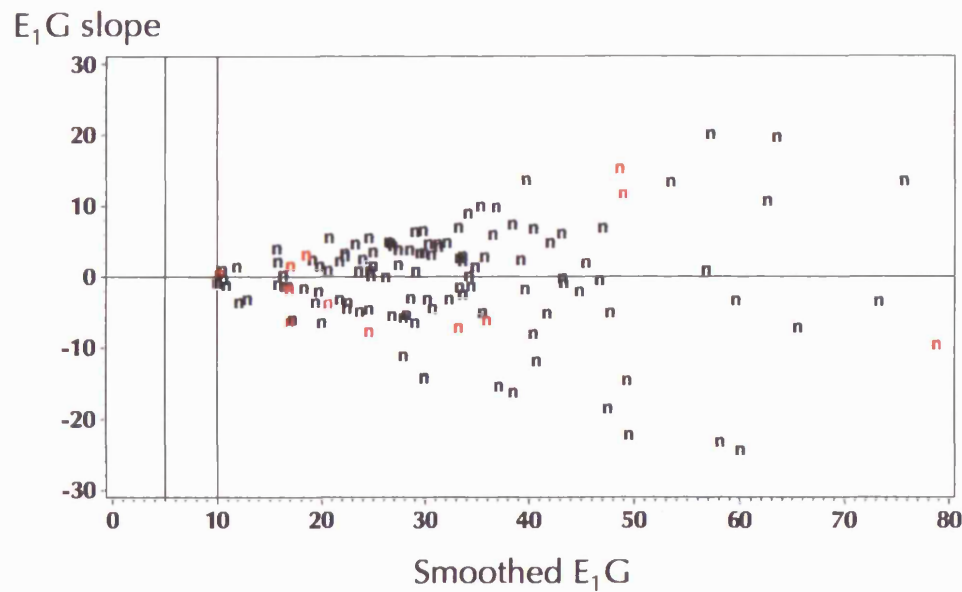


FIGURE 22: ANALYSIS OF RATE OF CHANGE AND CRITICAL THRESHOLD OF OESTROGEN

The data were also assessed for critical ‘threshold’ and rate of change of E₁G, both for individuals and as pooled data. Figure 22 shows an example of E₁G slope plotted against E₁G level data from one patient. Each point represents one day. If that day is a migraine event it is coloured red. If it is rate of change that matters migraine events would tend to fall below the slope = 0 line. If it is a critical threshold that matters then migraine events should tend to be on the left.

Analysis of pooled data did not reveal any relationship between the concentration of E₁G on the day on which migraine occurred in the late luteal phase, or on any of the five preceding days. Neither was there any apparent relationship between the rate of change of E₁G on the day on which migraine occurred in the late luteal phase, or on any of the five preceding days.

DISCUSSION

This study is the first to assess migraine during both rising and falling levels of oestrogen across the menstrual cycle. The strengths of this study are the careful diagnosis of migraine for each attack, the larger number of women, and daily hormone levels assessed over several consecutive cycles.

The results confirm previous studies showing that migraine is significantly more likely to occur in association with falling oestrogen in the late luteal/early follicular phase of the menstrual cycle. This supports the hypothesis of oestrogen 'withdrawal' triggering migraine. A new finding, which has not been assessed in earlier studies, is that migraine was significantly less likely to occur during phases of rising oestrogen. Although this study identified day -1 as the day of peak migraine incidence, day +1 was classified as the first FULL day of bleeding and in most cases menstruation had started on the day before. Hence these findings are compatible with the results of previous studies that identified peak migraine incidence at the start of bleeding.

Ovulation was confirmed by urinary hormone analyses. As in the diary card studies, there was no significant association between migraine and ovulation. Since only luteal oestrogen 'withdrawal' but not ovulatory oestrogen 'withdrawal' was associated with migraine, this supports the suggestion that a period of sustained high oestrogen priming is a necessary precursor.(Somerville, 1972b, 1975a, b)

The age of the study population is of note (mean age is 43 years). The clinical impression is that the menstrual trigger for migraine affects women entering the perimenopause more than younger age groups. Although there was no control population, this is supported by results of other studies of women not using hormonal contraception. In one study, the mean age of women in the menstrual migraine group was 37 years, whereas the mean age of women who had migraine unrelated to menstruation was 29 years.(Epstein *et al.*, 1975)

The failure to identify a critical 'threshold' for oestrogen associated with migraine in the late luteal phase is in line with the theory that falling levels are more important than absolute levels.(Somerville, 1972b) There was no evidence of an association between the rate of change of oestrogen 'withdrawal' and migraine. Other researchers have also failed to find a correlation with critical threshold or rate of change.(Martin *et al.*, 2005) However, they may still be important at an individual level although it would be necessary to have many more cycles of individual data to analyse this. Further, urinary E₁G levels may not be sensitive enough to establish a difference.

If oestrogen 'withdrawal' is an important mechanism, why does it not trigger migraine in all women? The mean peak luteal phase urinary E₁G level of 32.8 ng/mL is worth noting as it is significantly higher than the expected mean urinary peak luteal phase E₁G levels of 14.9 ng/mL for normal fertile cycling women reported in population studies matched by menopause status.(Miro *et al.*,2004b) Other researchers noted raised serum concentrations of both oestrogen and progestogen in women with migraine, most striking in the late luteal phase.(Epstein *et al.*, 1975) But high luteal phase oestrogen may just reflect the age of the population, as the perimenopause can be associated with high oestrogen levels.(Santoro *et al.*, 1996) High baseline oestrogen would result in a greater luteal drop, which might account for the increased prevalence of menstrual attacks of migraine at this time.

Biological predisposition may also be relevant. A study of postmenopausal women found that despite similar serum oestradiol levels, only postmenopausal women with a premenopausal history of migraine associated with menstruation developed migraine as oestrogen levels declined, following a single depot oestradiol injection.(Lichten *et al.*, 1996) In contrast, women with no premenopausal migraine did not develop migraine. This would be interesting to study in more depth in future research, particularly in light of a recent study of women with migraine and chronic daily headache, the results of which suggest that headaches are influenced by hormone fluctuations even in women without an apparent association with menstruation.(Martin *et al.*, 2003)

SUMMARY OF FINDINGS

In women diagnosed with menstrual migraine, migraine incidence was significantly higher during the late luteal/early follicular phase of falling oestrogen and significantly lower during rising phases of oestrogen, compared to the expected number of attacks. The early luteal post-ovulatory fall in oestrogen had no effect on migraine incidence. As previously reported, menstrual attacks were more severe and more likely to be associated with nausea or vomiting compared to non-menstrual attacks.

2.2. MIGRAINE AND OESTROGEN 'WITHDRAWAL' IN WOMEN USING COMBINED ORAL CONTRACEPTIVES: A REVIEW

Very little is known about the effects of combined oral contraceptives (COCs) on migraine. The methodology of studies assessing the effect of COCs on migraine has been inconsistent and few studies attempt to differentiate between headache and migraine.

HEADACHE AND COCS

Headache is a common side effect of COC use, with initial exacerbation in the early cycles of use followed by resolution with continued use.(Sluglett & Lawson, 1967; Brill *et al.*, 1990; Ernst *et al.*, 2002)

Of 3679 women starting COCs for the first time using 20µg ethinyloestradiol and 150µg desogestrel, 36% reported headache at baseline.(Ernst *et al.*, 2002) After three cycles, 14% reported that headache had worsened, 57% reported improvement and 28% reported no change. New onset of headache affected only 0.5%.

In a study of 3267 women starting a COC containing 30µg ethinyloestradiol and 75µg gestodene, 46% were first time COC users and 54% were switching from another brand.(Brill *et al.*, 1990) Of the 16% of women with headache at baseline, 63% reported improvement over the 18-cycle study. New onset of headache was reported by 8.8% of women in cycles 1 to 3, 3.9% in cycles 4 to 6, 3.9% in cycles 10-12, and 2% in cycles 16 to 18. Unfortunately, there was no analysis of new users versus switchers.

In addition, frequency of headache may depend on the type of progestogen and the dose of oestrogen used. Studies of COCs containing 30µg ethinyloestradiol and levonorgestrel, a second generation progestogen, noted headache in approximately 10% of all cycles.(Guillebaud, 1983) It might appear that use of COCs containing third generation progestogens is associated with headache as reviews of studies using COCs containing 30µg ethinyloestradiol and 150µg desogestrel found headache to affect only 5% of women during the sixth cycle.(Fotherby, 1995) However, this could be accounted for by differences in

study design as other studies have not found any differential effect from progestogens.(Cullberg, 1972; Dunson *et al.*, 1993; Koetsawang *et al.*, 1995)

Dose of ethinyloestradiol may be relevant as women using 20µg ethinyloestradiol and 150µg desogestrel reported fewer than 2% by the sixth cycle.(Fotherby, 1992)

MIGRAINE AND COCS

Few studies have specifically assessed migraine in COC users. The majority of publications assess high dose COCs containing at least 50µg ethinyloestradiol and pre-date the IHS diagnostic criteria. Migraine improves in a significant proportion of women and many report no change in migraine frequency or severity.(Whitty *et al.*, 1966; Phillips, 1968; Larsson-Cohn & Lundberg, 1970; Kudrow, 1975; Dalton, 1976; Ryan, 1978; Granella *et al.*, 1993; Cupini *et al.*, 1995)

As with headache, migraine, typically without aura, occurs during the hormone-free interval.(Whitty *et al.*, 1966; Phillips, 1968; Ryan, 1978; Horowski & Runge, 1986; MacGregor & Hackshaw, 2002)

The prevalence of headache and migraine among women using COCs was examined in a large, cross-sectional population-based study in Norway of 13,944 women.(Aegidius *et al.*, 2006) There was a significant association between use of COCs and migraine (30µg ethinyloestradiol OR 1.4 [95% CI 1.2 to 1.7], $P<0.001$) and for non-migrainous headache (30µg ethinyloestradiol, OR 1.2 [95% CI 1.0 to 1.4], $P=0.025$). In contrast, there was no significant association between progestogen-only pills and migraine (OR 1.3, 95% CI 0.9-1.8, $P=0.156$) or headache (OR 1.0 [95% CI 0.8-1.3], $P=0.1$).

A clinic based case-control study reviewed 39 women with migraine with aura and 83 women with migraine without aura who had used COCs.(Granella *et al.*, 2000) Migraine with aura was reported as worsening in 56.4% of cases compared to 25.3% of women with migraine without aura (OR 3.8 [95% CI 1.6-9.3]). There was no change in 38.5% of women with migraine with aura compared to 67.5% of women with migraine without aura (OR 0.3 [95% CI 0.1-0.7]). Improvement was reported by 5.1% of women with migraine with aura compared to 7.2% of women with migraine without aura (OR 0.7 [95% CI 0.1-4.1]).

A review of 36 women with migraine with aura and 86 women with migraine without aura using COCs attending a headache clinic noted 50% of women with migraine with aura reported worsening of migraine with COC use compared to 34.8% of women with migraine without aura.(Cupini *et al.*, 1995) There was no change in 27.7% of women with migraine with aura compared to 44.1% of women with migraine without aura. Improvement was reported by 0% of women with migraine with aura compared to 4.6% of women with migraine without aura. New onset of migraine was reported by 22.2% of women with migraine with aura and 16.2% of women with migraine without aura. In both groups, worsening during COC intake was more likely than improvement ($P<0.0001$).

HEADACHE AND MIGRAINE DURING THE PILL-FREE INTERVAL

If headache occurs, it is typically during the hormone-free interval.(Sulak *et al.*, 2000; LaGuardia *et al.*, 2005; Sulak *et al.*, 2007) These symptoms are most likely to result from withdrawal of ethinyloestradiol, similar to the oestrogen 'withdrawal' mechanism for menstrual migraine in the endogenous hormone cycle.

Although there are no published studies of migraine during the pill-free interval, these data provide circumstantial evidence for oestrogen 'withdrawal' as a putative mechanism.

2.3. CONCLUSIONS FROM PART TWO

The obvious hormones potentially involved in the pathophysiology of menstrual migraine are oestrogen and progesterone. A review of the literature suggested that the natural drop in oestrogen in the luteal phase of the menstrual cycles is associated with menstrual migraine.

From the data presented above, the following points can be concluded:

1. Compared to the expected number of attacks there was an inverse relationship between migraine incidence and oestrogen; the follicular phase rising oestrogen was associated with reduced risk of migraine and late luteal oestrogen 'withdrawal' was associated with increased risk of migraine.
2. Post-ovulatory falling oestrogen had no effect on risk of migraine.
3. An association between late luteal progesterone 'withdrawal' and migraine cannot be discounted, although there is evidence from published studies by other researchers to suggest that progesterone plays only a minimal part, if any, in menstrual migraine.
4. Although there are no studies on migraine, headache associated with combined hormonal contraceptive use typically occurs during the hormone free interval, providing circumstantial evidence for the role of oestrogen 'withdrawal' as a mechanism.

These findings support the hypothesis that menstrual migraine and headache during the hormone free interval of combined hormonal contraceptives are associated with oestrogen 'withdrawal'.

PART THREE:
MENSTRUAL MIGRAINE CAN BE PREVENTED WITH OESTRADIOL
SUPPLEMENTS

3.1. IDENTIFYING THE OPTIMAL DOSE AND TIMING OF OESTRADIOL SUPPLEMENTS FOR PREVENTION OF OESTROGEN 'WITHDRAWAL' DURING THE NATURAL MENSTRUAL CYCLE

3.1.1. IDENTIFYING THE OPTIMAL DOSE

A review of the literature confirms that stabilising oestrogen levels, either high or low, can prevent oestrogen 'withdrawal' migraine.

Somerville inhibited the endogenous menstrual cycle with 100mg oestradiol implants. The resultant fluctuating serum levels of oestradiol were associated with severe menstrual disturbance and worsening of migraine.(Somerville, 1975a). In contrast, Magos *et al.* showed that implant doses large enough to suppress ovulation and produce constant plasma oestrogen levels achieved a 96% response rate: 46% of women became completely headache-free, 37.5% reported almost complete symptomatic relief, and 12.5% reported partial benefit.(Magos *et al.*, 1983) However, such treatment is impractical for most women as it involves an (albeit minor) outpatient procedure and is not readily reversible. Implants slowly release oestradiol at constant levels over approximately six months although their effect can be sustained for up to two years. Further, progestogens, which are mandatory to prevent endometrial hyperplasia, can induce migraine.(Magos *et al.*, 1986)

Treatments that reduce oestrogen levels have been successful in the treatment of migraine: danazol is used to maintain steady, low levels of oestrogen; tamoxifen modifies the dynamics of oestrogen by its anti-oestrogen activity; and gonadotrophin releasing hormone analogues provoke a 'medical' menopause.(O'Dea & Davis, 1990; Holdaway *et al.*, 1991; Lichten *et al.*, 1991; Murray & Muse, 1997; Mathew & Fung, 1999)

An alternative strategy is to prevent the late luteal oestrogen fall using exogenous oestrogen perimenstrually, rather than throughout the cycle. There have been several small studies comparing oestradiol supplements with placebo. Those based on 25µg or 50µg oestradiol patches seemed to have little effect in reducing the occurrence of menstrual or menstrually-related migraine.(Pfaffenrath, 1993; Pradalier *et al.*, 1994; Smits *et al.*, 1994) However, there was a clear benefit in

trials using higher doses of 100µg oestradiol patches or 1.5mg oestradiol gel.(de Lignieres *et al.*, 1986; Dennerstein *et al.*, 1988; Pradalier *et al.*, 1994) The suggestion from these studies is that 25µg and 50µg oestradiol patches are not effective in preventing migraine as they result in suboptimal doses of oestrogen, achieving mean oestradiol serum levels of 25pg/ml and 40pg/ml respectively. In contrast, the 100µg oestradiol patch and 1.5mg oestradiol gel produce higher mean plasma oestradiol levels around 75-80pg/ml, equivalent to mid-luteal phase endogenous oestradiol levels.

On the basis of these findings, the optimal dose for perimenstrual prophylaxis appears to be either 100µg oestradiol patches, which are replaced every 3-4 days, or 1.5mg oestradiol gel, used daily.

3.1.2. IDENTIFYING THE OPTIMAL TIMING OF OESTROGEN SUPPLEMENTS

Having identified that oestrogen ‘withdrawal’ is associated with increased risk of migraine, the next step was to assess if migraine could be prevented with oestrogen supplements. However, to ensure the optimal outcome, timing of oestrogen needed careful consideration.

Author	Start of treatment	Type of oestrogen	Dose	Duration
Somerville (1972)	3-10 days before expected onset of menstruation	oestradiol valerate i.m.	5-10 mg in single or divided doses	N/A
de Lignières et al. (1986)	48 hours before the earliest expected onset of migraine	oestradiol gel	1.5mg	7 days
Dennerstein et al. (1988)	at 2 days prior to expected migraine	oestradiol gel	1.5mg	7 days
Pfaffenrath (1993)	2 days prior to suspected onset of migraine	oestradiol patches	50µg	not stated
Smits et al. (1993)	Day -2	oestradiol patches	50µg	8 days
Pradalier et al. (1994)	Day -4	oestradiol patches	25/100µg	8 days

TABLE 8: TIMING AND DOSE OF OESTROGEN SUPPLEMENTS FOR PREVENTION OF MENSTRUAL MIGRAINE

In previous studies (Table 8), oestrogen supplements have been timed based on the first day menstruation or the anticipated start of menstrual migraine, both of which are subject to variability and can be difficult to predict accurately.

This may not be the optimum time to bridge the late luteal drop in oestrogen, which starts from the time of peak luteal phase levels and continues until oestrogen levels start to rise in the follicular phase of the following cycle.

From Paper V data, peak luteal E₁G occurred on day -6 of the cycle and the follicular E₁G minimum was on day +2 of the cycle (Figure 23 and Figure 24).

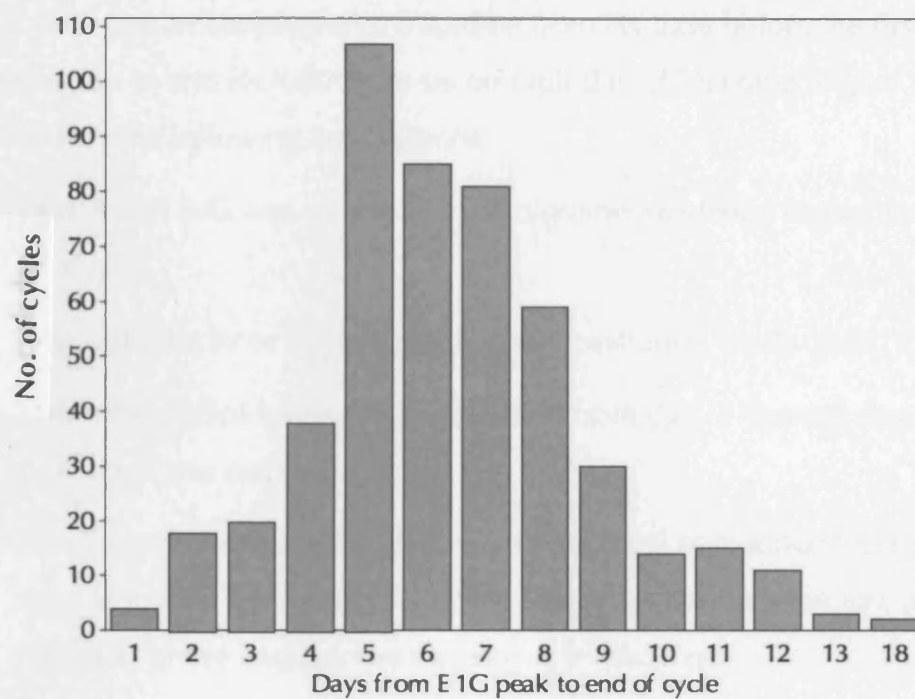


FIGURE 23: CYCLE DAY OF LUTEAL E1G PEAK N=38

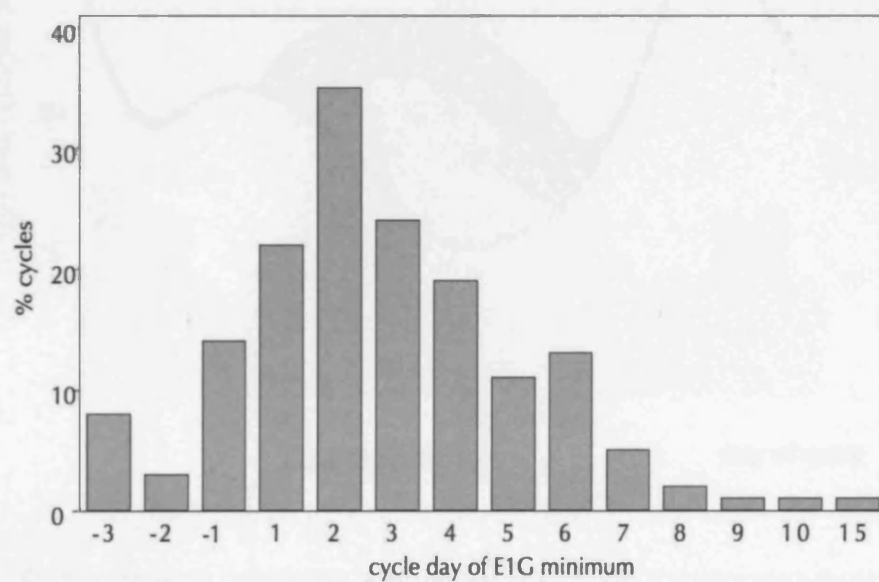


FIGURE 24: CYCLE DAY OF E1G MINIMUM N=38

To ensure adequate oestrogen levels over the 'prone' time we considered that the optimal time to start supplements would be from six days before the first full day of bleeding up to and including the second full day of bleeding (Figure 25). This was based on the following assumptions:

- Peak luteal E₁G was on day -6 and migraine incidence began to increase on day -3.
- It would take three days to reach stable oestradiol levels.(Kuhl, 1990)
- Stable oestradiol levels would continue from day -3 through day +2 when oestradiol was stopped.
- There would be a gradual decline in oestradiol over around three to five days following gel use.(Kuhl, 1990) This would allow sufficient time for follicular phase endogenous oestrogen levels to rise.

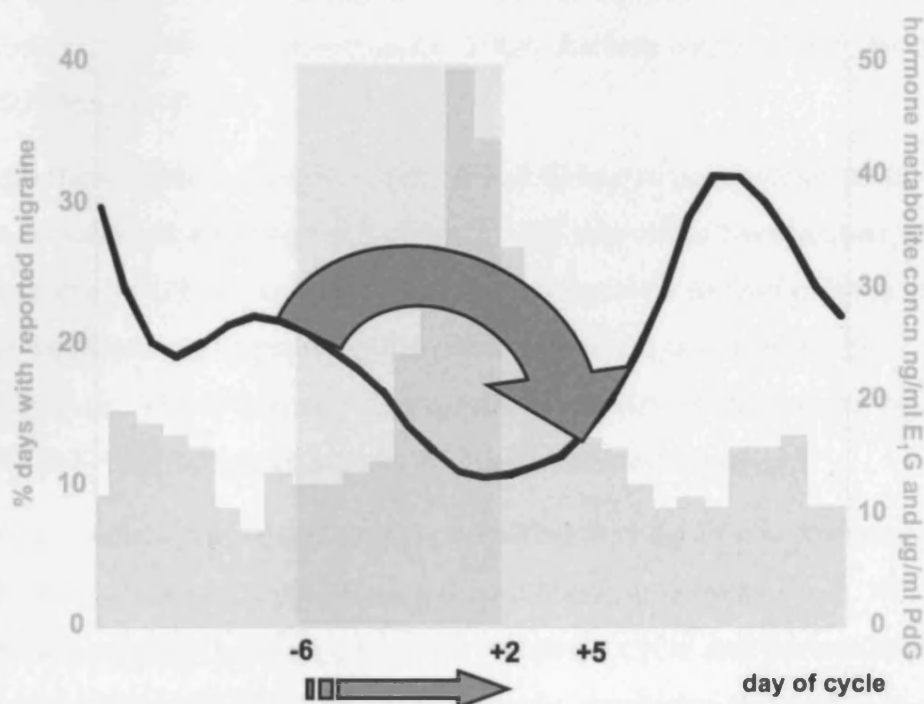


FIGURE 25: TIMING OF OESTRADIOL SUPPLEMENTS IN RELATION TO ENDOGENOUS OESTROGEN

The remaining problem was how to establish accurate timing of treatment.

3.2. PAPER VI: A METHOD TO PREDICT MENSTRUAL MIGRAINE AND OPTIMISE TIMING OF TREATMENT OF PERIMENSTRUAL PROPHYLAXIS

None of the published trials using premenstrual prophylaxis raised any concerns about the difficulty of timing treatment accurately.

Treatments have varied from use of standard prophylactics given perimenstrually such as pizotifen or propranolol, through to NSAIDs such as naproxen, and perimenstrual oestrogen supplements.(de Lignieres *et al.*, 1986; Dennerstein *et al.*, 1988; Sances *et al.*, 1990; Pfaffenrath, 1993; Pradalier *et al.*, 1994; Smits *et al.*, 1994; Kornstein & Parker, 1997)

More recently, interest has focused on perimenstrual prophylaxis with triptans, originally developed for the acute treatment of migraine. Sumatriptan, naratriptan and frovatriptan have been studied for perimenstrual prophylaxis, starting treatment 2-3 days before the expected onset of migraine.(Newman *et al.*, 1998; Newman *et al.*, 2001; Silberstein *et al.*, 2004; Tuchman *et al.*, 2005; Mannix *et al.*, 2007; Brandes *et al.*)

Although these studies did not report correct timing of prophylaxis as an issue, the patient populations entering such clinical trials may differ from women seeking treatment in clinical practice. For example, many of these studies have included women experiencing migraine in the pill-free interval of combined oral contraceptives. These women will experience regular 28-day cycles, so that it is easy to predict the timing of attacks and perimenstrual treatment.

In clinical practice, the largest group consulting for migraine is women in their late 30s or 40s, who are approaching the menopause.(Stewart *et al.*, 1991) Given the normal variation in the length of the menstrual cycle and the variation in the timing of menstrual attacks from cycle to cycle, predicting the time to start prophylaxis can be difficult.

Further, patient recall of menstrual cycles and migraine correlates poorly with objective information.(Waters & O'Connor, 1971; Lipton *et al.*, 1992; MacGregor *et al.*, 1997) Although diary cards can resolve this problem in part, the natural

variation in cycle length within individual women can mean that identifying the optimum time to start premenstrual prophylaxis is somewhat hit-and-miss.

Reviewing 492 menstrual cycles from 31 women participating in clinical trials of a triptan for perimenstrual prophylaxis, cycle length varied by 9.9 ± 7.5 days.

Timing of prophylaxis was accurately predicted in 63/232 (26.7%) cycles of treatment. The discrepancy of actual versus predicted menstruation was 1.9 ± 2.5 (range -12 to 17) days (unpublished data).

The peak time for migraine during the menstrual cycle is on or between two days before the start of menstruation and the first three days of bleeding. (Waters & O'Connor, 1971; Dalton, 1973; MacGregor *et al.*, 1990; Johannes *et al.*, 1995; Stewart *et al.*, 2000; MacGregor & Hackshaw, 2004) Therefore, rather than predicting the expected day of migraine in order to commence perimenstrual prophylaxis, a more sensible approach would be to predict the first day of menstruation and time prophylaxis relative to this, continuing treatment throughout the vulnerable period.

Menstruation consistently occurs close to 14 days following ovulation. (Speroff & Fritz, 2004) On this basis, some studies have measured the ovulatory rise in temperature in order to predict ovulation and time perimenstrual prophylaxis. (Szekely *et al.*, 1989) Temperature can be affected by many factors, not least use of analgesics for migraine, which can inhibit the small postovulatory rise in basal body temperature. Another indicator of ovulation is the change in the quantity and quality of cervical mucous (Billings method). (Billings, 1964) These two methods are often combined so that a deficiency in one method is balanced by the advantage of the other, thereby resulting in greater efficiency. However, the chore of observations and charting is an unwanted inconvenience for many women and long-term compliance is unlikely.

A simple method that predicts menstruation reliably would enable many women with pure menstrual and menstrually-related migraine to predict menstrual attacks and time perimenstrual prophylaxis more accurately, over consecutive cycles of treatment.

The Clearblue Easy Fertility Monitor (Unipath Diagnostics Inc, Waltham, MA) is a home ovulation test that simultaneously detects luteinizing hormone (LH) and oestrone-3-glucuronide (E₁G) in early morning urine, thus identifying the likely time of ovulation.(Behre *et al.*, 2000)

To test the accuracy and acceptability of this device in migraine, a trial was undertaken using the monitor to predict the start day of perimenstrual oestrogen prophylaxis in women with menstrual migraine.

OBJECTIVES

The objectives of this study were to assess the accuracy and the suitability of the fertility monitor in the management of menstrual migraine by:

- Assessing the accuracy of ovulation prediction using the monitor compared to urinary hormone analyses;
- Assessing the accuracy of using a simple algorithm to calculate optimal timing of perimenstrual supplements for the prevention of menstrual attacks of migraine;
- Assessing patient acceptability of the monitor.

METHODS

The trial was conducted following Good Clinical Practice (GCP) and with the Declaration of Helsinki (1964), as amended in South Africa (1996). All study documents were approved by an independent Ethics Committee.

INCLUSION AND EXCLUSION CRITERIA

Diagnosis of migraine with and without aura was in accordance with IHS criteria.(Headache Classification Committee of the International Headache Society, 1988)

Pure menstrual migraine was defined as migraine on or between days –2 to +3 (assuming day 1 is the first day of menstruation and that there is no day 0) in at least 2/3 cycles with no migraine at other times of the cycle.

Menstrually-related migraine was defined as up to four attacks of migraine per month of which one must occur on or between days –2 to +3 in at least 2/3 cycles.

Eligibility was based on diary-card review to confirm pure menstrual or menstrually-related migraine. Women were not eligible if they: had headaches/migraine more often than three days a week; used symptomatic medications regularly on more than three days a week; were pregnant, or intended to become pregnant during the study (women used non-hormonal contraception); were breastfeeding; had impaired liver or kidney function; had polycystic ovarian syndrome; used any hormonal treatment within the previous six months; used tetracycline; consumed a high soy diet or took soy/isoflavane supplements; had any medical or psychiatric condition that might be affected adversely by oestrogen supplements or which would preclude participation.

INTERVENTIONS

Eligible women who gave informed consent were given a monitor, test sticks, and packs for daily urine collection. They kept headache diary cards, recording symptoms and treatments taken. After three baseline cycles, women used perimenstrual oestradiol gel or placebo for six treatment cycles.

Home study procedures

Urine samples

The first morning urine samples were collected in universal vials containing sodium azide (0.1%), a preservative.

Fertility monitor

The hand-held monitor (Figure 26) comes with disposable dual-assay urine test sticks and is designed for use by women with cycle lengths of 21-42 days. It detects ovulation by tracking changes in urinary levels of two female sex hormones in early morning urine:

- oestrone-3-glucuronide (E₃G), levels of which correspond to plasma levels of oestradiol with a rise during the follicular phase of the menstrual cycle triggering a surge in luteinizing hormone.
- luteinizing hormone (LH), the surge of which occurs 24-36 hours prior to ovulation.(Behre *et al.*, 2000)

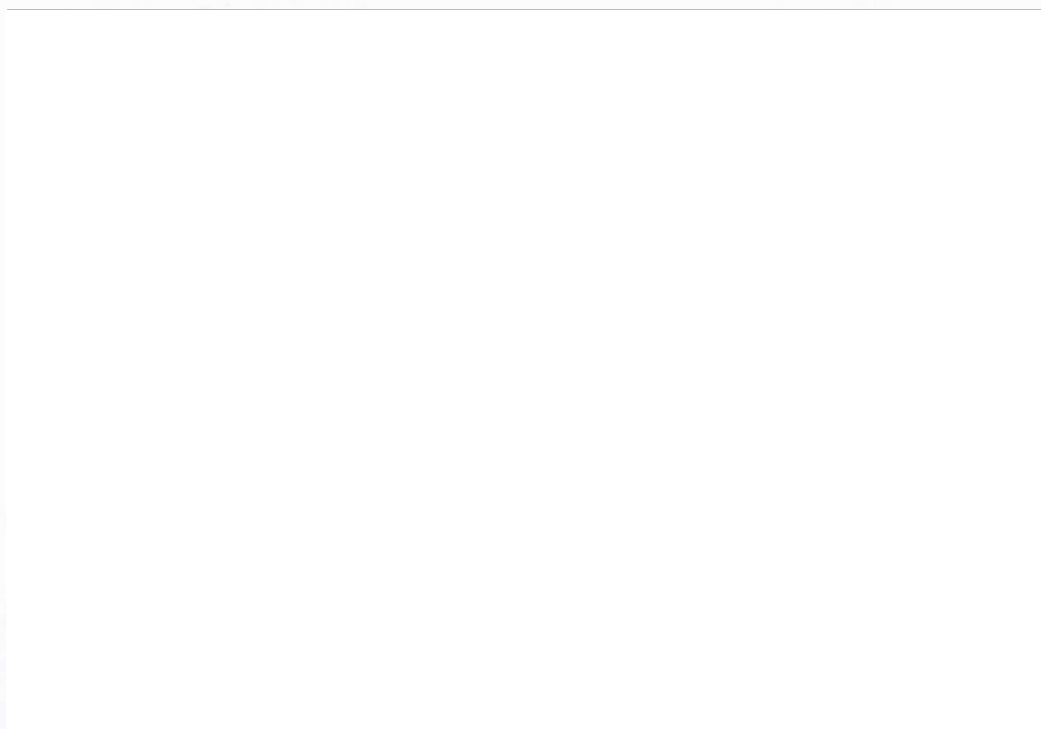


FIGURE 26: THE CLEARBLUE EASY FERTILITY MONITOR (UNIPATH DIAGNOSTICS INC)

On the morning after the start of the first period after entering the study, each woman pressed the 'm' button to initiate that cycle of use. The women were instructed to look at the monitor every morning and, through its display, the monitor guided them through each cycle (Figure 27). The monitor requires one test, using a disposable dual-assay urine stick, every day for 10 to 20 days, depending on the length of the menstrual cycle and the timing of the LH surge. The test stick is held for 3 seconds in the collected urine sample and inserted into the monitor, which displays the result within five minutes. In the first cycle, the first test is requested on day 6. In subsequent cycles the monitor calculates the start day depending on the accumulated information about the individual user's cycles.

Oestradiol and placebo gel

Women calculated the start day of treatment by counting the first day that they saw the peak fertility symbol as Day 1. They were instructed to use the gel on Day 10 (first day of LH surge + 9 days) continuing until the second full day of bleeding, i.e. day 2 of the next cycle.

Monitor display:

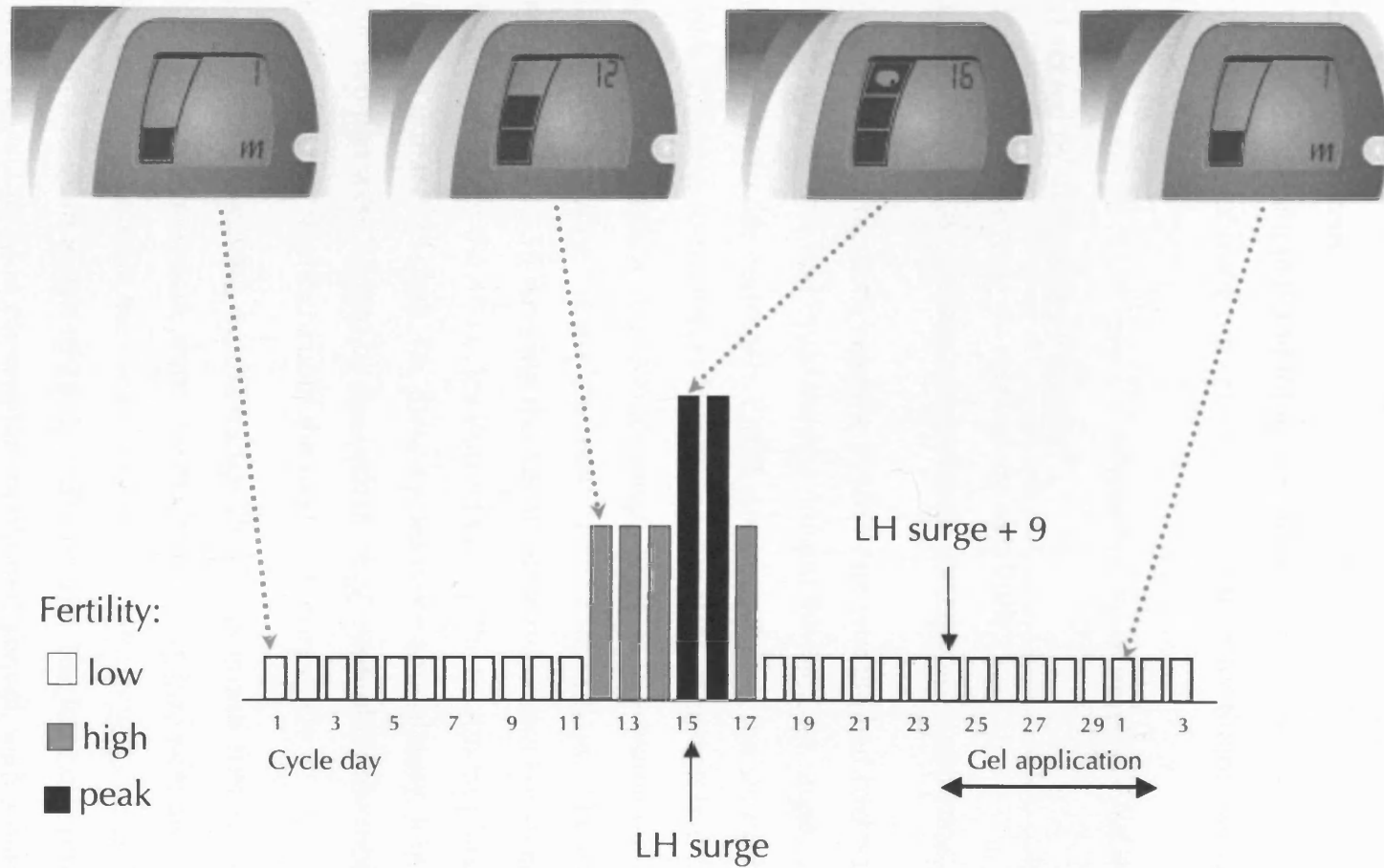


FIGURE 27: GRAPHICAL REPRESENTATION OF USING THE FERTILITY MONITOR TO TRACK LUTEINIZING HORMONE (LH) SURGE AND START OF TREATMENT

Laboratory study procedures

Women sent their urine samples to the laboratory each week. Samples were analyzed in duplicate for E₁G, LH, pregnanediol 3-glucuronide (PdG) and follicle-stimulating hormone (FSH).

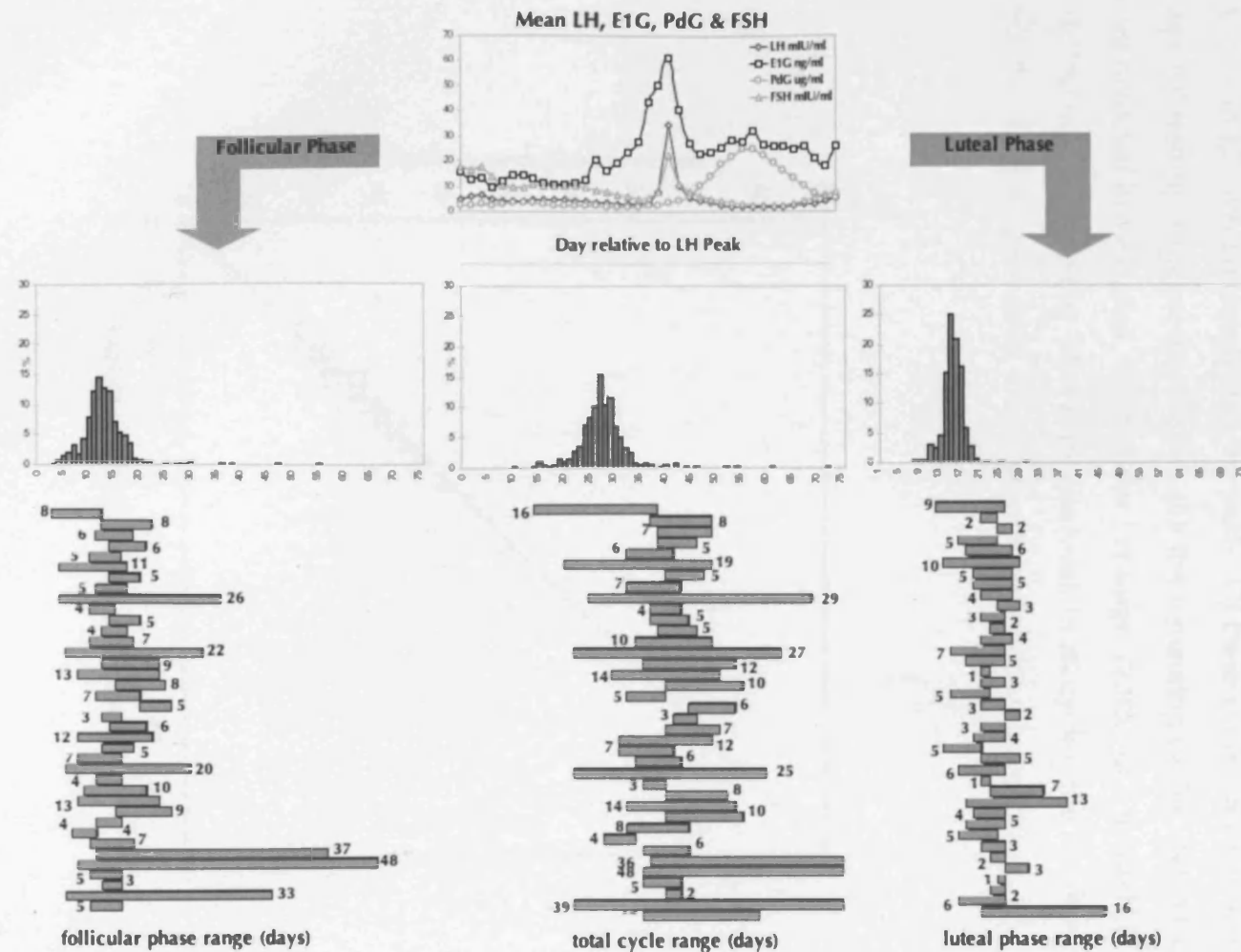
Outcomes assessed were:

- The duration of luteal and follicular phases;
- The accuracy of using the LH surge as a marker for timing oestradiol intervention;
- The percentage of LH surges confirmed by laboratory analysis that were detected by the Fertility monitor;
- Subjective comments on method acceptability.

RESULTS

Of 40 women recording three baseline cycles, one woman had had no menstrual attacks of migraine, one had cycle lengths outside the monitor range, and one withdrew consent to use oestrogen. Of 37 women entering the six-cycle treatment phase: one withdrew to conceive; four had incomplete data; and five had cycle lengths consistently outside the monitor range. In these five women, cycle lengths of ovulatory cycles during the study ranged from 15 to 72 days. The follicular phase ranged from 4 to 55 days but the luteal phase was more constant, ranging from 10 to 19 days. Of the 46 cycles studied during the treatment phase, 17 (37%) had no urinary LH peak, i.e. these cycles were anovulatory. If ovulation did not occur, women were alerted by the lack of 'egg' symbol on the monitor that cycle and so deferred treatment until the next ovulatory cycle.

The mean age of the women was 43 (range 29-50) years and 30% were over 45. During the six-cycle treatment phase the cycle length in one woman ranged from 15 to 42 days but otherwise the cycle length of ovulatory cycles ranged 20 and 37 days, with a median length of 28 days (Figure 28). The follicular phase ranged from 7 to 22 days (5 to 22 in the woman mentioned above), with a median length of 15 days. The luteal phase was more consistent within individual women, ranging from 7 to 18 days in all 27 women, with a median length of 14 days. Of the 221 cycles during treatment, 29 (14%) were anovulatory.



Top: mean hormone levels across the cycle. Middle: percentage of women (y-axis) versus cycle length in days (x-axis). Bottom: follicular phase length, total cycle length and luteal phase length (days). Horizontal bars represent each individual n = 38: min (range) max

FIGURE 28: CYCLE LENGTHS

In all but two women the algorithm of the LH surge + 9 days accurately calculated 6 days before the first day of menstruation in the majority of cycles. These two women had consistently short luteal phases necessitating a different algorithm.

With respect to the LH surge, analyses were undertaken on the 182 ovulatory cycles in 27 women completing the study. Of these cycles, 8 were outside the normal testing range for the monitor. Of the remaining cycles, the LH surge was not detected in 17 cycles, due to poor LH surge (2.9%) or a missed LH surge (6.7%) (as confirmed by laboratory analysis). In all cycles, the monitor detected 90.2% of LH surges within the range of the monitor (Figure 29).

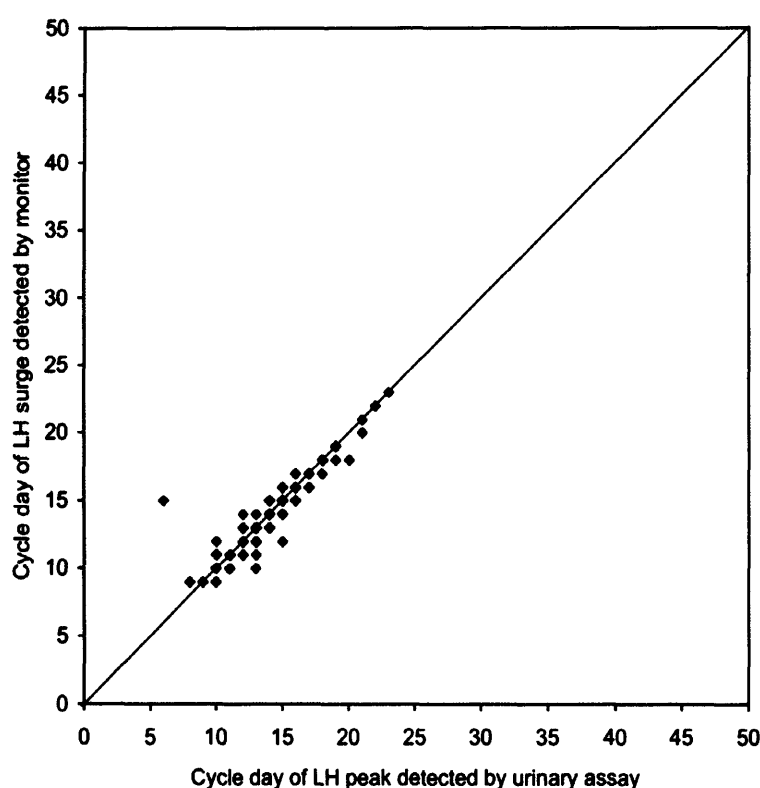


FIGURE 29: MONITOR PERFORMANCE: LH SURGE CYCLE DAY (MONITOR) VS. LH PEAK CYCLE DAY (URINE ANALYSIS). LH, LUTEINIZING HORMONE

Regarding method acceptability, 35 women responded to a questionnaire inviting comments about the study (APPENDIX 2). Thirty-three women (94%) found the monitor useful and provided the following comments:

"I found I could judge the 'danger period' for migraine very accurately"

"You could adjust your life-style because you had more of an idea when migraine would occur... it helped plot more accurately [than diaries]"

"I found just being totally tuned in to where my body was in its monthly cycle helped me to 'manage' attacks. For instance, I felt more confident as to whether I could or could not risk a glass of wine!"

77% of women wanted to continue using the monitor as a treatment aid:

"I missed the monitor a lot when the trial ended - slightly irregular cycles mean I have to guess a lot..."

"Very easy to use - it became part of my daily routine, I even used it camping!"

"Useful to remind me IF I should be taking oestrogen gel" (woman with some anovulatory cycles)

DISCUSSION

The monitor was easy to use and accurately identified ovulation, enabling prediction of menstruation and timing of perimenstrual migraine prophylaxis. This was despite variation in cycle length, which largely resulted from variation in follicular phase length; following ovulation, the luteal phase was relatively constant. The monitor was designed for use by women with cycle lengths of 21 to 42 days, which led to exclusion of five women with cycle lengths outside this range. For such women, using test sticks every day of the cycle until ovulation rather than selected days, would circumvent this problem.

Because the luteal phase is consistently 14 days, the treatment algorithm correctly predicted 6 days before the onset of menstruation in 25 of the 27 women completing the study per-protocol. Two women had short luteal phases and the algorithm had to be modified. In practice, written instructions could enable women to calculate their own algorithm. The software could easily be modified to calculate the algorithm automatically, based on data collected about the users' own luteal phase, showing a symbol to identify the start day of perimenstrual prophylaxis.

For women treated by a health-care professional there is also the option to download the monitor's memory onto a small data card for transfer to a home computer for display and storage. Data cards can also collect information on events, such as migraine attacks.

When using oestrogen supplements, the benefit of confirming ovulation is that women can avoid using oestrogen supplements in anovulatory cycles, in which no progesterone is produced. These cycles are more common in women approaching the menopause and the 'unopposed' endogenous oestrogen increases the risk of endometrial hyperplasia and cancer.

The monitor can be used to time non-hormonal and even non-drug treatments used perimenstrually. Predicting menstruation also enables women to avoid additional migraine triggers around their 'prone' time and has obvious advantages for early intervention with symptomatic treatments.

SUMMARY OF FINDINGS

A home-use fertility monitor was used to time perimenstrual prophylaxis in women with menstrual or menstrually-related migraine. There was considerable inter- and intra-variability in cycle length, mostly due to follicular phase differences; the post-ovulatory luteal phase was relatively constant. The monitor accurately identified ovulation in over 90% of cycles, enabling prediction of menstruation and precise timing of perimenstrual prophylaxis. Ninety-seven percent of women found the monitor useful in predicting menstrual migraine attacks.

3.3. PAPER VII: PREVENTION OF MENSTRUAL MIGRAINE WITH PERIMENSTRUAL OESTRADIOL

This study aimed to assess the effect of perimenstrual oestradiol on the incidence of menstrual migraine when used in conjunction with the fertility monitor. By identifying peak fertility associated with ovulation, the monitor could predict menstruation and hence be used to indicate when to apply the gel. (MacGregor *et al.*, 2005; Behre *et al.*, 2000) Further, by collecting early morning urine, the effect of oestradiol supplements on migraine could be correlated with changes in hormone levels both during and after use, which has not been assessed in previous studies. The effect of oestradiol on the length of the follicular and luteal phases was also examined.

OBJECTIVE

Based on the hypothesis that migraine attacks in the late luteal phase are associated with oestrogen 'withdrawal', the objective of the study was to assess the effect of perimenstrual percutaneous oestradiol on migraine in the late luteal phase of the menstrual cycle in women with menstrual or menstrually-related migraine. The main outcome was migraine occurrence defined as the number of days on which there was a migraine attack.

METHODS

Subjects

Women attending the City of London Migraine Clinic and who had participated in the earlier observational study of the association between oestrogen and migraine (Paper V) were invited to participate in this randomised placebo-controlled crossover study. They were eligible for inclusion if they had pure menstrual migraine or menstrually-related migraine, and had regular menstrual cycles (21-35 days). Women who had additional non-migraine headaches could participate provided that they could distinguish these headaches from migraine. Non-hormonal migraine prophylaxis could be continued provided that the dose remained stable during the study period.

Women were not eligible for the study if: headaches and/or migraine occurred more often than three days a week on average; analgesics or acute headache

medications were used regularly on more than three days a week; they were pregnant, or intended to become pregnant during the study period, or were breastfeeding; had evidence of impaired liver or kidney function; had evidence of polycystic ovarian syndrome (unless they had regular menstrual periods); used hormonal contraception, hormone replacement therapy or other hormonal treatment within six months prior to the start of the study or at any time during the study period; used treatments that might affect the menstrual cycle such as tetracycline, a diet high in soy, or use of soy/isoflavane supplements; or had any medical or psychiatric condition that might have been affected adversely by use of oestrogen supplements or which would preclude participation in the study. All women were required to provide informed consent.

Definitions

Menstrual migraine was defined as migraine attacks on or between day 1 of menstruation \pm 2 days (i.e. on or between days -2 to $+3$ of the cycle assuming day 1 is the first day of menstruation and that there is no day 0) in at least two out of three cycles with no migraine at other times of the cycle. Menstrually-related migraine was defined as up to four attacks of migraine per month of which one must occur on or between day 1 of menstruation \pm 2 days in at least two out of three cycles.

Migraine data

Migraine data, as it occurred, was recorded on a diary card for each month, and included date and time of onset of symptoms; peak severity (mild, moderate or severe); duration of attack (to nearest day); associated symptoms (nausea, photophobia and phonophobia); aura, if present; medication (name, dose, time taken).

Adverse events

Adverse events were recorded on the diary cards as they occurred. If women were concerned about any unusual symptoms that they experienced, they were instructed to contact the clinic or telephone a 24-hour emergency contact number.

Fertility Monitor

The system comprises a hand-held monitor (Figure 26) and disposable dual-assay urine test sticks and was designed for use by women with cycle lengths of 21-42 days (Clearblue Easy Fertility Monitor - Unipath Diagnostics Inc., Waltham, MA). The test sticks simultaneously detect luteinizing hormone (LH) and oestrone-3-glucuronide (E₁G) in early morning urine, which identify ovulation. This has been previously described in detail.(Behre *et al.*, 2000) Women conducted a test each day as requested by the monitor by using a sample of early morning urine. By this method the women identified the day of LH surge in each cycle by the appearance of the peak fertility symbol on the monitor screen enabling them to calculate when to start using the perimenstrual supplements. Each woman performed at least 10 tests per cycle.

Urine Samples

Women collected early morning urine (EMU) samples on each morning of the study. Samples were collected in universal vials containing sodium azide (0.1%) as a preservative. During the collection period, women sent their samples to the laboratory weekly. At the laboratory, urine specimens were aliquoted and stored at 4°C until analyses were carried out.

Hormonal Analyses

Samples were analysed for oestrone-3-glucuronide (E₁G) and pregnanediol 3-glucuronide (PdG), which are the urinary metabolites of oestradiol and progesterone, together with luteinizing hormone (LH), and follicle-stimulating hormone (FSH) using an AutoDelfia® (Perkin Elmer Life Sciences, Cambridge, UK). LH was assayed to confirm ovulation and to compare urinary analyses with monitor performance, reported in a separate study. FSH was assayed and, together with the other hormones, enabled classification of menopause status.

Analyses were conducted with highly specific in-house developed antibodies, as previously described.(Miro *et al.*, 2004c) A standard curve comprising six standards (in triplicate) and three quality control samples were run for each assay. Twenty-five replicates of each standard were run as samples and the concentration of each was determined using Multicalc®. The lowest detectable

concentration of the assays was determined to be 0.09 IU/L (LH); 0.17 IU/L (FSH); 0.1 ng/ml (E₁G); and 0.07 µg/ml (PdG). Samples were assayed in duplicate.

Urinary E₁G reflects the changes of plasma oestradiol (E₂). (Catalan *et al.*, 1989) Early morning urine (EMU) samples were convenient and the excretion pattern of E₁G in EMU is similar to the pattern obtained for 24-hour urine samples. (World Health Organization, 1983) Because of this, concentrations can be expressed in mass/volume instead of mass steroid/creatinine ratio. (Adlercreutz *et al.*, 1982) Further, there is evidence that creatinine adjustment is not essential for the study of menstrual cycle urinary hormones based on daily sampling. (Miro *et al.*, 2004c) The urinary data were shifted back one day for comparison with the diary card data, since morning collection of E₁G and PdG reflect serum hormone levels 12-24 hours earlier. (Munro *et al.*, 1991)

Oestrogen supplements

The dispensing pump delivers unit doses of 0.5g of gel containing 0.5mg of oestradiol. Three pump doses (1.5mg oestradiol) were used daily applied to the upper arms (avoiding the breasts) or thighs, on a large clean dry area of skin that had not been covered with any creams or talcum powder. This could be morning or evening, providing that this timing was consistent throughout the study. Women were advised to wait one or two minutes before covering the area with clothing.

Each woman was given oestradiol gel for three menstrual cycles and placebo gel for three cycles, in a crossover trial. Supplements were started six days before the first full day of bleeding up to and including the second full day of bleeding.

The time period from the LH surge to menses is consistently close to 14 days in ovulatory cycles. (Speroff & Fritz, 2004; MacGregor *et al.* 2005) The monitor indicated the LH surge with a specific symbol representing peak fertility. Women calculated the start day for the gel by marking on their diaries the first day that they saw the peak fertility symbol on the monitor as day 1. They were instructed to start using the gel on day 10 (day of LH surge + 9 days), approximating to six days before the first full day of bleeding, continuing to use it daily until, and including, the second full day of bleeding of the next cycle. Since the monitor collected information within a time window in the morning only, if a period started later in

the day the first cycle day would be recorded on the morning of the next day. To check the correct order was followed, the women were asked to write the code of the gel allocated for that cycle on the diary card.

The incidence of short luteal phases in the normal population is about 5-6%.(Lenton *et al.*, 1984) In line with this, two women were identified with short luteal phases in the preliminary study and the timing of oestradiol gel was adjusted accordingly (six and eight days following the LH surge, respectively).

All women were instructed to use the gel only if they saw the peak fertility symbol. Hence gel was only used during ovulatory cycles, ensuring endometrial protection from endogenous progesterone during treatment.

Compliance

On completion of, or following withdrawal from the study, all unused supplies were accounted for and returned to the clinic. Although the urinary E₁G levels could test compliance with the active gel, they did not ensure compliance with placebo gel. Therefore we weighed the gel containers when they were dispensed and when they were returned to ensure that the same amount had been used from each container.

Blinding and randomisation

Oestradiol and matching placebo were packaged identically and the two gels were indistinguishable. Each woman received six gel packs, labelled A to F. Unipath Limited undertook blinding so that neither the investigators nor the women knew which was oestradiol and which was placebo. Women were randomly allocated to receive either oestradiol or placebo for their first cycle, and then given in an alternating order for the remaining five cycles. However, since the gels were identical and each woman received six different packs neither the patient nor researcher would have been able to predict the next treatment. There was also unlikely to be any carryover effect between the gels because there was a sufficiently long length of time between stopping one gel and starting the next (31 days on average; 5-95th centile 16-75 days). Even if there were some carryover effect this could only be from using the oestradiol gel before the placebo gel, which would dilute any treatment effect.

Sample size

The sample size was calculated based on each woman providing data for three placebo cycles and three oestradiol cycles. Previous data showed a mean of 3.5 migraine days per cycle per woman with a standard deviation of 1.5.(MacGregor & Hackshaw, 2004) Assuming that there would be 10 migraine days over three cycles using placebo, a study of 36 women would have 90% power to detect a 25% reduction in migraine days (from 10 to 7.5), at the 5% level of statistical significance.

Statistical analysis

Each woman recorded the start and end date of each gel. To assess the effect of oestradiol compared to the placebo we compared the proportion of days on which a migraine occurred when the woman was on oestradiol with the proportion of migraine days when she was on placebo, and estimated the difference (i.e. a within-woman comparison). This was analysed, as in a standard crossover trial, with allowance for a possible period effect - that is, whether the effect on migraine was influenced by the ordering of the oestradiol and placebo gels.(Armitage & Berry, 1987)

We also estimated the relative risk, namely, the percentage reduction in migraine days per woman when using the oestradiol gel compared to placebo. For each woman the relative risk was estimated using the ratio of the proportion of days on which a migraine occurred (oestradiol versus placebo), and then pooled across all women using an exact method.(Martin & Austin, 2000) The analysis based on relative risks did not allow for an ordering effect of the gels, although as shown in the Results section, there was no evidence of such an effect.

The effect of the oestradiol gel was assessed for all women who had used at least one cycle of oestradiol and one of placebo. Data were also analysed for the subset of women who had regular menstrual cycles and had used all six gels in accordance with the protocol (referred to as “per-protocol” population). The analyses of urinary hormones and length of the follicular and luteal phases were restricted to the per-protocol population.

Cycles between LH peaks were defined, and split into luteal and follicular phases by the first day of bleeding. Where an LH peak was not defined the data between neighbouring LH peaks was removed. For the analysis of luteal phase length, 'oestradiol' and 'placebo' corresponded to gel application in that phase. For the analysis of follicular phase length 'oestradiol' and 'placebo' corresponded to gel application in the preceding luteal phase. Two-way analysis of variance was used providing a within-woman comparison.

RESULTS

Of 38 women eligible to participate in the crossover study, 37 received study treatment and one woman withdrew from the study before treatment allocation (Figure 30). Two women used only one gel for one cycle and, therefore, did not provide any useful information for a within-woman analysis.

Data were analysed for the remaining 35 women, 31 used all six allocated gels (three oestradiol and three placebo), two women used four gels (two oestradiol and two placebo), one woman used three gels (one oestradiol and two placebo) and one woman used two gels (one oestradiol and one placebo).

Of these 35 women, urinary hormone data for four women showed elongated cycles with evidence of delayed ovarian response indicative of the perimenopause.(Miro *et al.*, 2004b) Two women had missed treatment cycles, one had started gel early, and one had several days of missing urine samples. Hence data for 27 women who had used all six gels in accordance with the protocol were available for per-protocol analysis.

Patient characteristics are shown in Table 9 and Table 10.

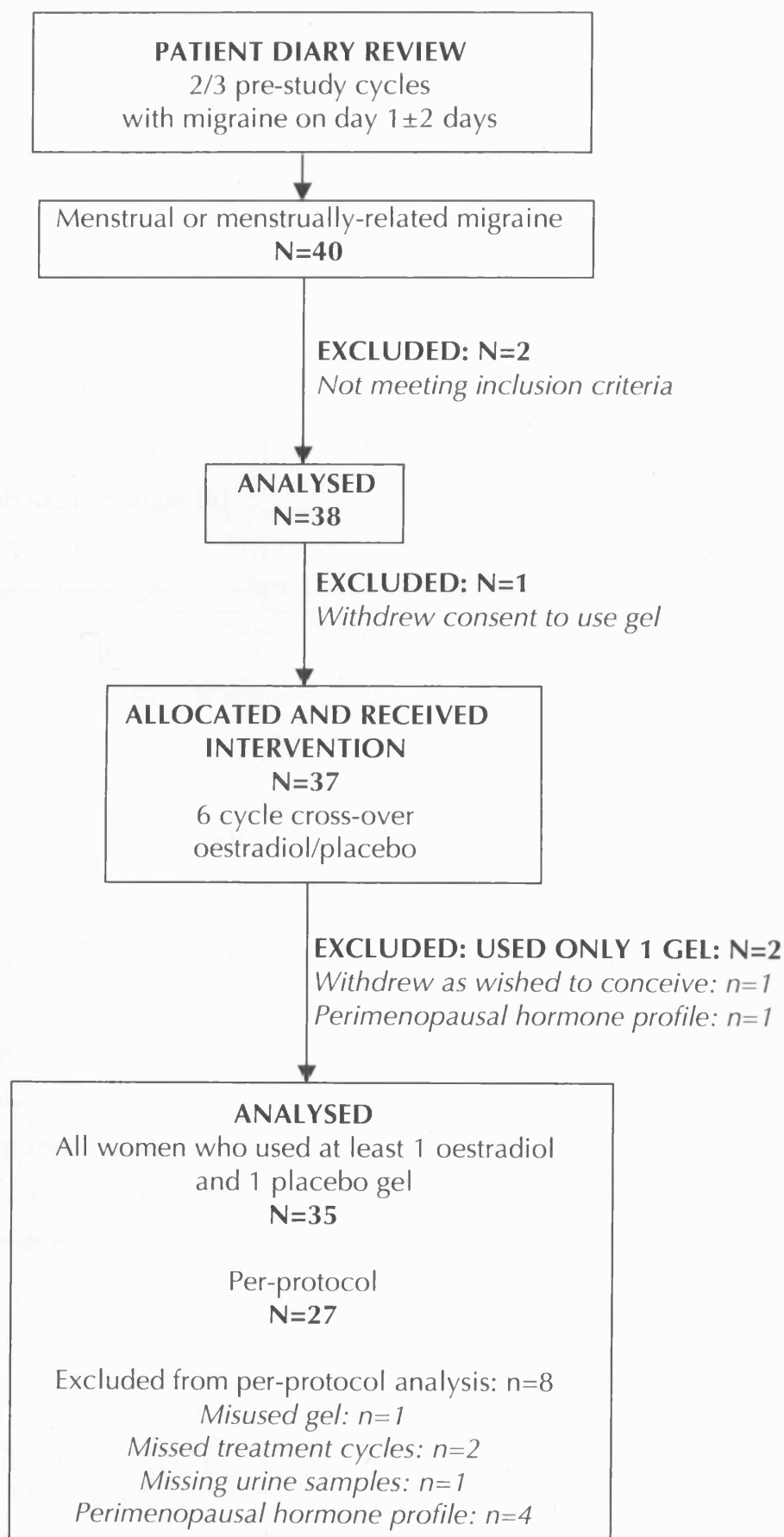


FIGURE 30: PARTICIPANT FLOW

	All women recruited (n=38)	All women in the crossover study (n=35)	Per protocol (n=27)*
Mean age (years)	43	43	43
Range (years)	29-50	29-50	29-50
Over 45 (%)	29	29	30
Over 35 (%)	87	86	89
Type of headache (n)			
Migraine without aura	37	34	26
Migraine with & without aura	1	1	1
Association with menstruation (n)			
pure menstrual migraine	1	1	1
menstrually-related migraine	37	34	26

*women with regular menstrual cycles who used all 6 gels in accordance with the protocol

TABLE 9: SELECTED PATIENT CHARACTERISTICS

	All women recruited (n=38)	All women in the crossover study (n=35)	Per protocol (n=27)*
Acute treatment (n)	38	35	27
analgesics only	9	8	7
triptans only	6	5	4
ergots only	1	1	0
analgesics and triptans	21	20	15
analgesics and ergots	1	1	1
Prophylactic treatment (n)	9**	9**	7**
amitriptyline	4	4	2
beta-blocker	4	4	4
fluoxetine	1	1	1
pizotifen	2	2	2

*women with regular menstrual cycles who used all 6 gels in accordance with the protocol

** 2 women each took two prophylactic agents

TABLE 10: MIGRAINE DRUGS USED BY WOMEN DURING THE STUDY

Effect of oestradiol gel on migraine frequency and severity subsequent to gel use

In all, there were 133 migraine days whilst women were using oestradiol and 171 when using placebo, a difference that is statistically significant ($P=0.03$). The within-woman analysis based on the difference in the proportion of gel-days on which a migraine occurred (active vs placebo gel), after allowing for a possible period effect, yielded a difference in percentage points of -3.6% [95% CI -8.8 to 1.6%], in favour of the oestradiol gel. The difference was almost statistically significant ($P=0.08$). There was no evidence of an effect of the order of oestradiol and placebo gels ($P=0.43$).

Figure 31 shows the individual relative risk of having a migraine day using oestradiol compared to placebo for each of the 35 women.

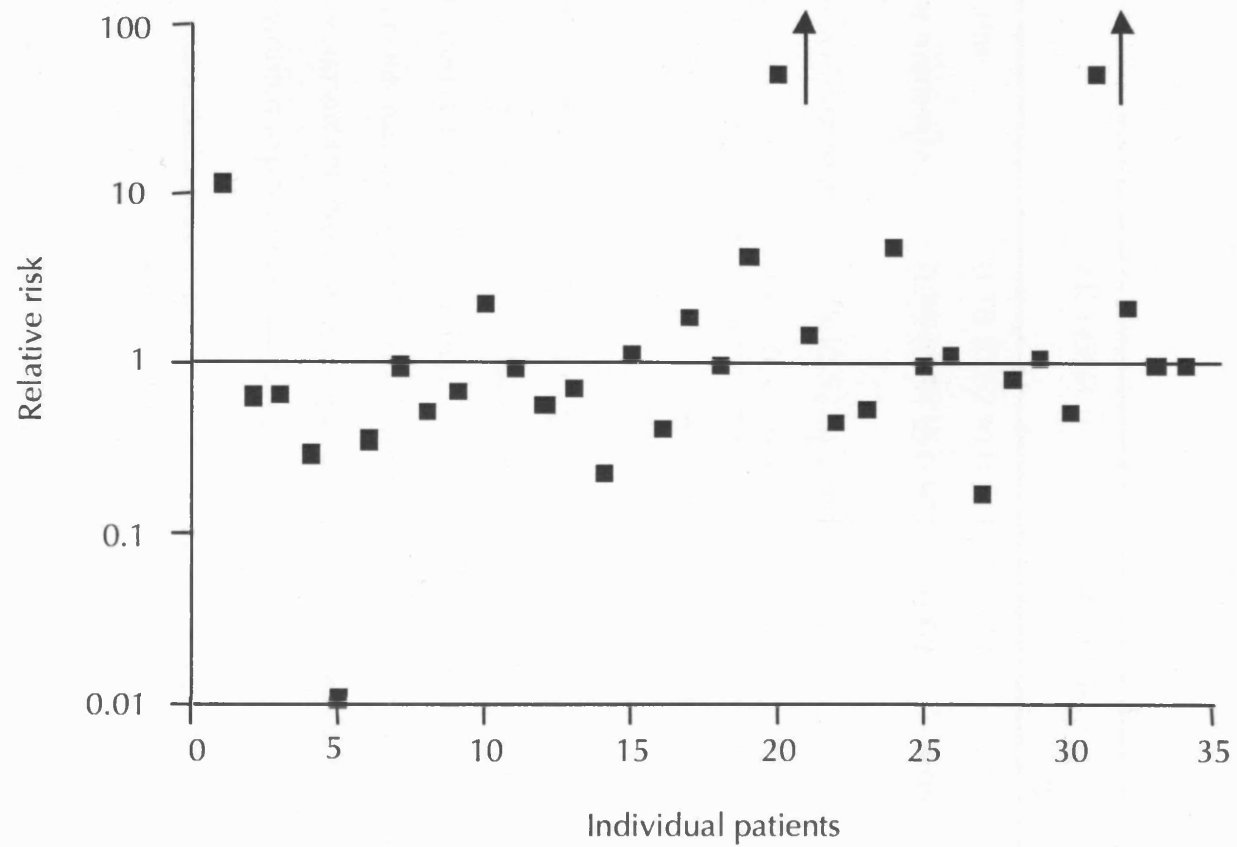


FIGURE 31: RELATIVE RISK (RR) OF MIGRAINE USING OESTRADIOL GEL VS. PLACEBO GEL (N=34)

Table 11 shows the average relative risk for the group of 35 women. Oestradiol was associated with a statistically significant 22% reduction in migraine days per woman; RR 0.78 [95% CI 0.62 to 0.99]. These attacks were also less severe ($P=0.03$) and there was evidence that they were associated with less nausea, although this difference was not statistically significant. The effect was similar if the data were restricted to the per-protocol population ($n=27$) there was a 22% reduction in migraine days (RR 0.78 [95% CI 0.60 to 1.01], $P=0.06$).

	RR [95%CI]	<i>P</i> -value	Total no. of migraine days
All migraine	0.78 [0.62 to 0.99]	0.04	304
Severe or moderate migraine	0.73 [0.54 to 0.97]	0.03	206
Migraine with nausea	0.75 [0.52 to 1.06]	0.10	139
Migraine with vomiting	0.86 [0.33 to 2.19]	0.90	22

TABLE 11: RELATIVE RISK (RR) OF MIGRAINE USING OESTRADIOL VS. PLACEBO

Table 12 shows the effect of gel on migraine occurring on days 1 ± 2 of the cycle in all women. The results are consistent with those in Table 11, in that oestradiol was associated with a reduction in migraine days. However, since the time period is narrower, the results are based on fewer events and the difference was not statistically significant. No results are presented on migraine with vomiting since only four women experienced vomiting.

	RR [95%CI]	<i>P</i> -value	Total no, of migraine days
All migraine	0.85 [0.58 to 1.43]	0.42	120
Severe or moderate migraine	0.77 [0.48 to 1.24]	0.31	78
Migraine with nausea	0.93 [0.49 to 1.76]	0.92	44

TABLE 12: RELATIVE RISK (RR) OF MIGRAINE ON DAY 1 ± 2 USING OESTRADIOL VS. PLACEBO

There was, however, evidence of an increase in migraine occurrence in the five days immediately following oestradiol use compared to placebo, RR 1.40 [95% CI 1.03 to 1.92], $P=0.03$. The relative risk of having a moderate or severe attack was 1.40, [95% CI 0.97 to 2.05] but this was not statistically significant, $P=0.07$. Of the 22 women who benefited from using the oestradiol gel (they had fewer migraines compared to using placebo), 15 experienced post-gel migraine (they had more migraine days during the 5 days after the oestradiol gel compared to the 5 days after placebo). This effect appeared to disappear after 5 days; the relative risk of having a migraine between 5 and 10 days after gel use was 1.04 [95% CI 0.67-1.62], $P=0.92$.

Effect of oestradiol gel on urinary hormones

Oestradiol gel increased basal E_1G , delaying the E_1G fall in some cases until early in the subsequent cycle. Figure 32 shows the overlaid oestradiol levels from active versus placebo or untreated cycles from one woman. This gives an example of the effect of gel on urinary E_1G , with the 'double hump' created by endogenous luteal phase E_1G followed by a rise in E_1G associated with exogenous gel use, and subsequent delay in oestrogen 'withdrawal'. A further example from a single cycle is shown in Figure 33.

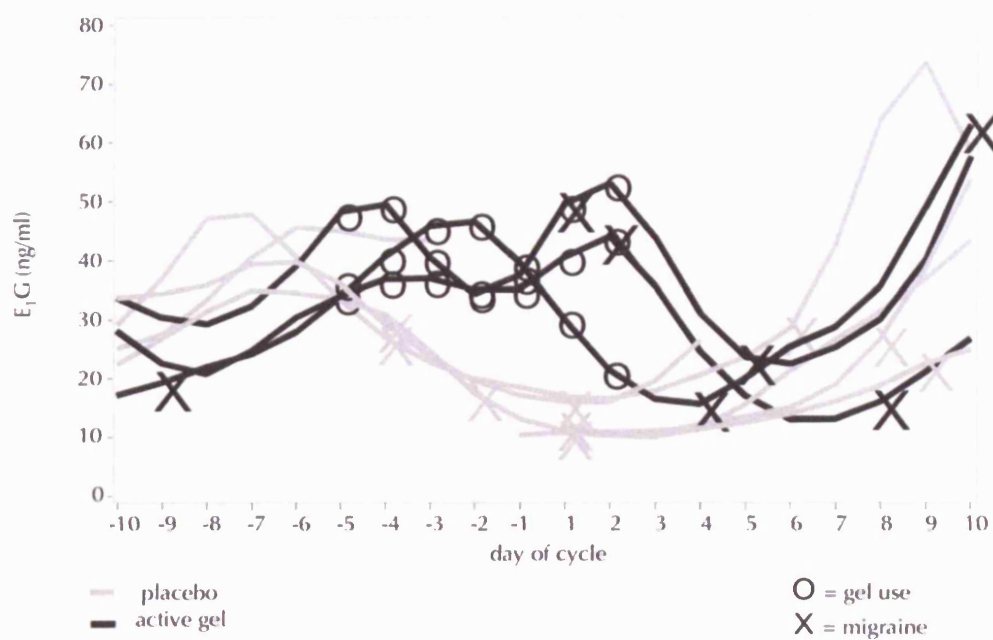


FIGURE 32: SMOOTHED E_1G LEVELS AND MIGRAINE ATTACKS DURING PLACEBO AND ACTIVE GEL CYCLES IN ONE VOLUNTEER

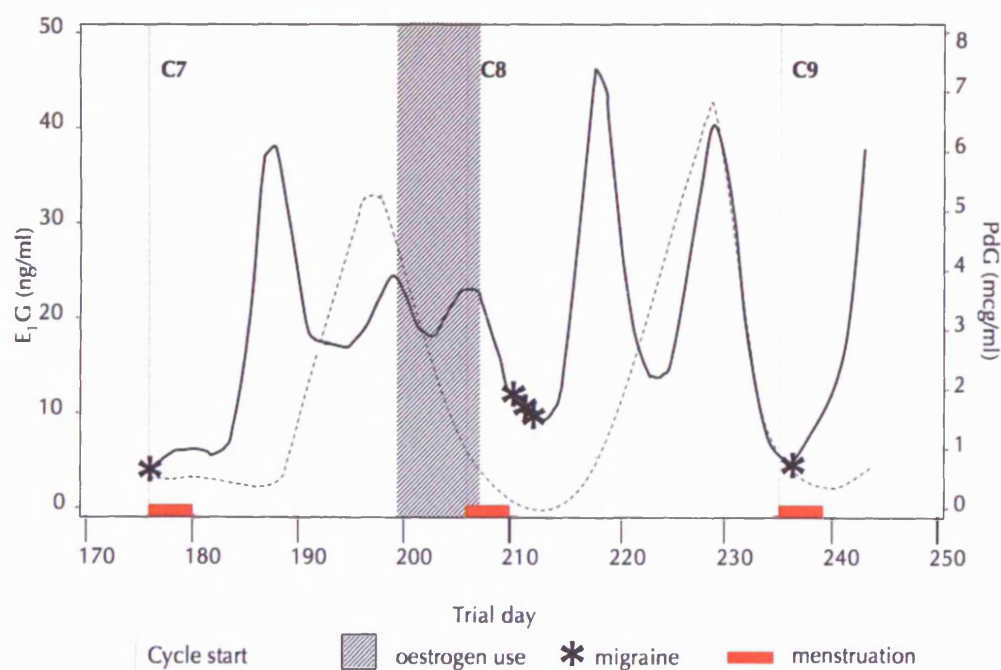


FIGURE 33: EXAMPLE OF 'DOUBLE' HUMP OF URINARY E_1G ASSOCIATED WITH USE OF OESTROGEN GEL

The E₁G concentration following the end of gel use is shown in Figure 34. E₁G levels remained elevated above those associated with placebo cycles for four days, a difference that was significant for the first three days ($p < 0.0001$). The mean difference from placebo cycles was 16ng/ml and the mean rate of decline of E₁G was 4ng/ml per day.

Peak incidence of migraine was 3 days following the end of gel use is shown in Figure 35.

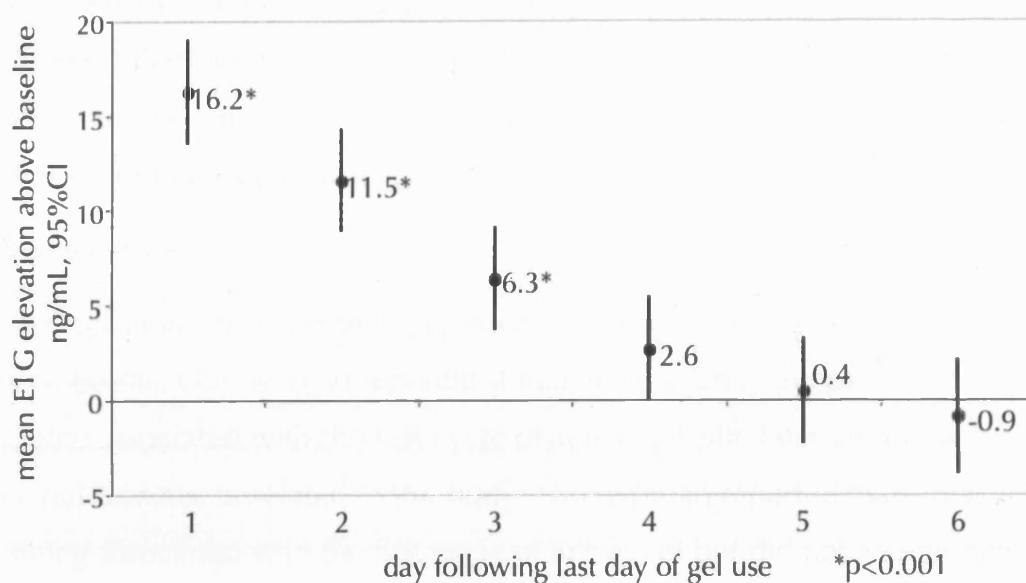


FIGURE 34: DIFFERENCE BETWEEN MEAN E₁G LEVELS ON OESTRADIOL GEL COMPARED TO PLACEBO GEL FOLLOWING THE LAST DAY OF GEL USE; PER-PROTOCOL POPULATION (N=27)

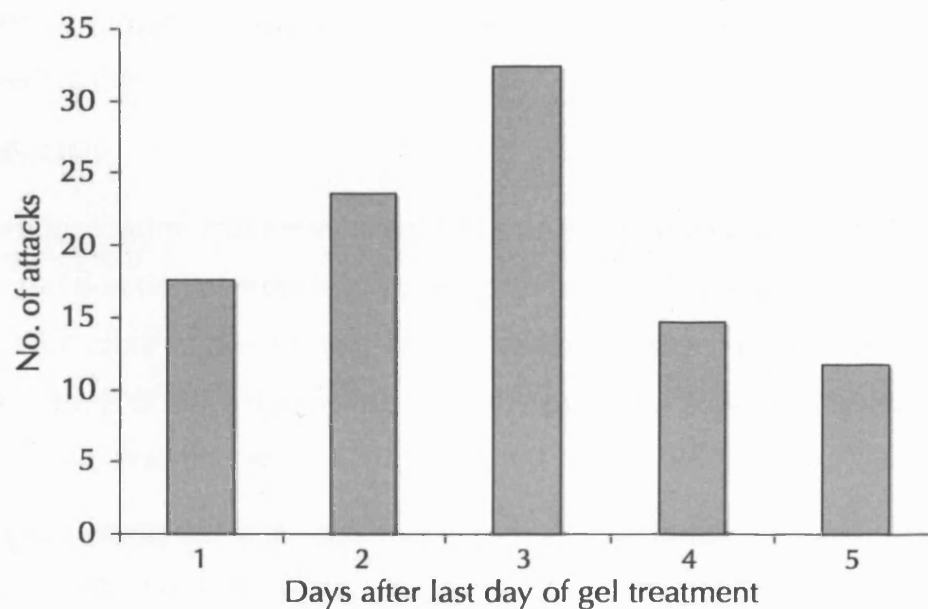


FIGURE 35: INCIDENCE OF MIGRAINE FOLLOWING THE LAST DAY OF GEL TREATMENT (N=21)

Effect of oestradiol on the length of the follicular and luteal phases

Oestradiol was associated with a significant increase in the length of the follicular phase (mean 13.90 days [95% CI 13.25 to 14.54] compared to placebo (mean 12.51 days [95% CI 11.85 to 13.16], $P=0.003$ versus oestradiol). Oestradiol had no effect on the length of the luteal phase (mean 14.26 days [95% CI 13.89 to 14.63] for oestradiol; mean 14.46 days [95% CI 14.09 to 14.84] for placebo).

Adverse events

No serious adverse events were reported. There were two possible treatment-related events. One woman reported stomach pains and nausea unrelated to migraine associated with the first cycle of active gel; she later withdrew for personal reasons, unrelated to the study. One woman reported diarrhoea and vomiting associated with the first cycle of active gel but did not experience similar symptoms with the other two cycles using active gel.

Compliance

Compliance throughout the study was excellent with no women lost to follow-up. There were missing urine samples for only 2.6% of days over the course of the study. There were no discrepancies in the weights of gels returned compared to the expected weights following gel use, confirming compliance with use of the study medication.

DISCUSSION

These results confirm that perimenstrual oestrogen supplements can reduce the severity and duration of menstrual attacks of migraine during treatment. This is in keeping with other studies using a similar dose of oestrogen to achieve serum oestradiol levels of at least 60pg/mL. (Dennerstein *et al.*, 1988; de Lignières & Bousser, 1992; Pradalier *et al.*, 1994)

If oestrogen withdrawal is the *sole* mechanism of 'menstrual' migraine, stabilising oestrogen levels should be 100% effective for these attacks. The fact that this treatment does not produce such a favourable outcome could be due to several reasons:

- The dose of oestrogen used failed to achieve stable plasma levels. This is important as menstruating women may produce a surge of endogenous oestrogen over and above the levels of oestrogen maintained by the exogenous treatment. Such fluctuations may be sufficient to trigger migraine.
- Only one patient had pure menstrual migraine in this study. Compared to pure menstrual migraine, menstrually-related migraine is only partially hormone dependent and may be less responsive to oestrogen treatment. (Dennerstein *et al.*, 1988)
- Other mechanisms (hormonal and/or non-hormonal) are acting in addition to oestrogen withdrawal.
- Oestrogen withdrawal is not the direct mechanism of 'menstrual' migraine.

However, the benefits are offset by an increase in migraine following gel use, associated with an iatrogenic delayed oestrogen 'withdrawal'. Reviewing the previous studies, Somerville identified delayed migraine in single cases over single cycles. (Somerville, 1972b, 1975a, b) De Lignières noted only one of eighteen patients had migraine three days after stopping oestradiol. (de Lignieres *et al.*, 1986) Other clinical studies have not commented on this, possibly because the post-treatment phase was not analysed since data only compared the time periods during which oestradiol or placebo oestrogen was applied. (Dennerstein *et al.*, 1988; Pradalier *et al.*, 1988; Pfaffenrath, 1993; Smits *et al.*, 1994)

Possible reasons for the occurrence of post-gel oestrogen 'withdrawal' migraine are that:

- The dose of oestradiol was inadequate.
- The duration of treatment was too short and, in some women, oestradiol treatment was stopped before follicular oestrogen had started to rise; had active treatment been continued until follicular-phase oestrogen levels were rising, it is possible that post-treatment headaches might have been avoided.
- Exogenous oestrogen inhibited the follicular rise of endogenous oestrogen. This latter possibility is consistent with the observation that the follicular

phase was extended by over 1 day in oestradiol treated cycle. This might be tempered by tapering the dose of exogenous oestrogen gradually over several days into the early follicular phase.

It seems unlikely that the dose used was inadequate, provided that the women used it correctly. Women were instructed to use the gel anywhere below the waist and, at the first visit, were shown the approximate size of area to cover. Variation in the area of gel application could have a significant effect on serum oestradiol levels.(Kuhl, 1990) Compliance checks, weighing the containers, suggested that the correct amounts of gel were used. A review of the individual graphs of E₁G levels (APPENDIX 3) shows that most women did achieve the 'double-hump' although not always consistently. Further, the dose used in this study was the same dose that had been effective in earlier studies.(de Lignieres *et al.*, 1986)

The duration of use of oestrogen supplements in the trial had been based on the expected perimenstrual increase in migraine, starting two days before the onset of menstruation.(MacGregor, 1996) Supplements were started around six days before the first full day of menstruation, coinciding with the mean luteal E₁G peak and allowing three days to achieve steady state levels. Based on our findings, tailoring the start of oestrogen supplements to each individual, based on an analysis of individual variation in the day on which menstrual attacks occur would be optimal.

Extending the duration of use of oestrogen supplements until endogenous oestrogen had risen might prevent post-supplement oestrogen 'withdrawal' migraine. In this study, oestradiol was used until day 2 of the cycle based on analysis of the pre-treatment study cycles, which showed that median cycle day of the E₁G nadir was day 2. However, there was wide inter- and intra-individual variation and in some women the E₁G nadir did not occur until day 6 or even later (Figure 36). In such cycles, post-gel oestrogen 'withdrawal' would not be tempered by endogenous oestrogen and could account for the post-gel increase in migraine. Similar to tailoring the start day of oestrogen supplements, future studies could tailor the duration of oestrogen supplements to individual variation in cycle day of the E₁G minimum.

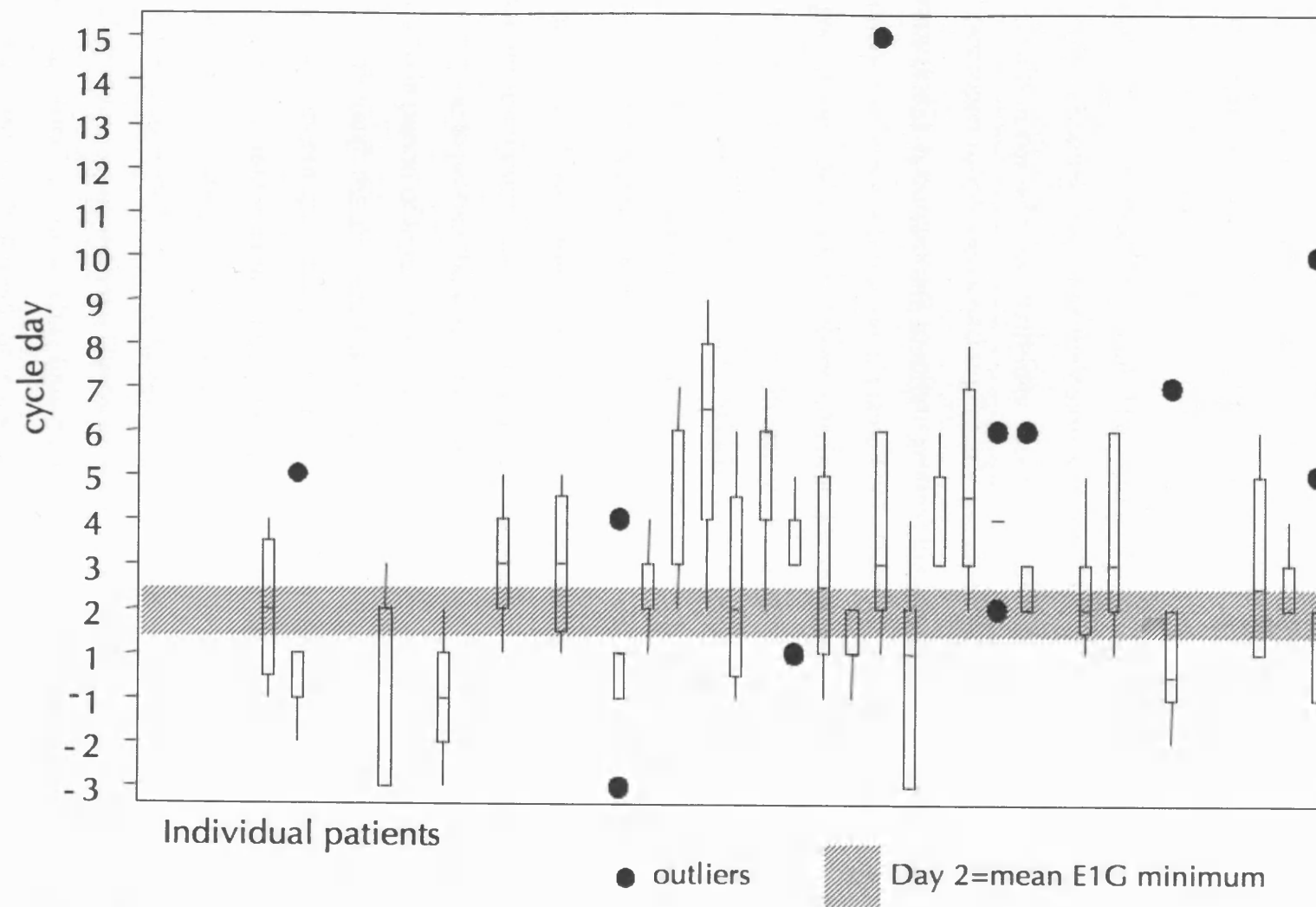


FIGURE 36: BOX PLOTS SHOWING VARIATION OF LATE LUTEAL/EARLY FOLLICULAR E₁G MINIMUM IN EACH PATIENT OVER THREE PRE-TREATMENT CYCLES

It is interesting to note that post-treatment headache has been reported in trials of perimenstrual prophylaxis with triptans. Two randomized controlled trials of naratriptan for short-term prevention of menstrual migraine, reported significant rebound headache.(Mannix *et al.*, 2007) This has not been seen in similar trials with frovatriptan. This may in fact be related to differences in pharmacokinetic properties, particularly since frovatriptan has a significantly longer half-life than all other triptans.(Buchan *et al.*, 2002)

Compliance in our study was high. This reflects the interest and commitment of the women hoping to identify the cause and potential treatment of a condition that results in considerable morbidity. However, some women were concerned that oestrogen supplements had similar adverse effects as hormone replacement therapy (HRT). It is important to inform women that supplementing oestrogen perimenstrually is not the same as taking HRT. There is no evidence that use of exogenous oestrogen, even if given continuously in higher doses necessary for contraception, increases the risk of breast cancer when given to premenopausal women.(Marchbanks *et al.*, 2002) Similarly, there is no evidence that HRT increases the risk of breast cancer when used premenopausally by menstruating women with a normally timed menopause.

A more relevant risk is the potential effect of oestrogen supplements on the endometrium if used during anovulatory cycles, during which there is no progesterone to protect the endometrium. If 'unopposed' oestrogen is continued over a long period of time, there is increased risk of endometrial hyperplasia and cancer, although the absolute risk of acquiring these disorders is small. This is of particular concern given the age of women presenting with menstrual or menstrually-related migraine, as typified in this study. The specific strengths of the present study are the population size, daily hormone analysis over several cycles, confirmed diagnosis for each migraine attack, accuracy of diary card data, and use of within-woman analysis. The main potential weakness is that women attending a specialist headache clinic might not represent the general population of women requiring treatment for menstrual attacks of migraine. However, previous studies in both community and clinic based populations have shown a

similar prevalence of menstrual migraine. (MacGregor *et al.*, 1997; Couturier *et al.*, 2003)

Future studies should assess the optimal dose and duration of oestrogen supplements to avoid deferring oestrogen 'withdrawal' migraine.

SUMMARY OF FINDINGS

Percutaneous oestradiol was associated with a 22% reduction in migraine days (RR 0.78 [95%CI 0.62 to 0.99], $P=0.04$); these attacks were less severe and less likely to be associated with nausea. This was followed by a significant 40% increase in migraine in the 5 days following oestradiol compared to placebo (RR 1.40 [95%CI 1.03 to 1.92], $P=0.03$).

Although perimenstrual percutaneous oestradiol showed benefit during treatment, this was offset by deferred oestrogen withdrawal after the gel was stopped, triggering post-dosing migraine.

3.4. PREVENTION OF OESTROGEN 'WITHDRAWAL' MIGRAINE IN WOMEN USING COMBINED HORMONAL CONTRACEPTIVES

3.4.1. PREVENTION OF MIGRAINE IN THE HORMONE-FREE INTERVAL OF COMBINED HORMONAL CONTRACEPTIVES: A REVIEW

If oestrogen withdrawal is a mechanism for menstrual migraine, it follows that attacks of migraine during the hormone-free interval of combined hormonal contraceptives (CHCs) might be associated with a similar mechanism and thus be prevented with oestrogen supplements.

Few studies have assessed this strategy. One study, published in 1968 as a letter, attempted to treat headaches (not distinguished from migraine) in the pill-free interval of combined oral contraceptives (COCs), using oral ethinylloestradiol.(Eichner, 1968) Improvement was seen in 34 of 50 women given 5-20µg ethinylloestradiol during the pill-free interval but there was no response to placebo. No comment was made on whether one dose was more effective than any other dose. However, the study is subject to a number of criticisms, not least because it was neither randomised nor double-blind.

An open-label study of 11 women using 0.9mg conjugated equine oestrogens on each day of the pill-free interval of a COC containing 20µg ethinylloestradiol reported a mean 77.9% reduction in the number of headache days.(Calhoun, 2004)

Other studies of headache in the hormone-free interval have achieved similar outcomes by replacing the 21/7 regime with an extended or continuous regime. A randomized clinical trial was conducted with 239 women at nine clinical research sites to compare bleeding profile, headache frequency, and subject satisfaction with the transdermal contraceptive (norelgestromin/ethinylloestradiol transdermal system) used in an extended regime (84 days) with a traditional, 28-day cyclic regime. In a majority of women studied, compared with cyclic use, extended use of transdermal norelgestromin/ethinylloestradiol delayed menses and reduced the total incidence of mean headache days during the hormone-free interval.(LaGuardia *et al.*, 2005)

In an open label single-centre prospective study, standard 21/7 cycles of a COC containing 3mg of drospirenone and 30µg of ethinyloestradiol were followed by a 168-day continuous regime. Of the 114 patients who began the trial, 111 completed the 21/7-day cycle portion of the study. Based on the headaches scales, there were significant differences in headache severity among the 28 days of the standard 21/7 cycles ($P < 0.001$). Greater headache severity occurred on days 25 through 28 during the 7-day placebo interval of the 21/7 cycles ($P < 0.05$). Of the 111 patients who completed the 21/7 phase of the study, 102 (92%) completed the 168-day continuous COC regime. Women with higher total headache scores demonstrated a significant reduction in daily headaches beginning in the first 28 days of continuous pill-taking ($P < 0.0001$), which persisted throughout entire 168 days. In contrast, headache in women with lower total headache scores remained unchanged throughout the continuous regime ($P = 0.79$). Impact of headaches on work, family, and social functions also improved on the continuous regime in six of eight measures assessed by weekly headache questionnaires ($P < 0.05$). (Sulak *et al.*, 2007)

CURRENT PRACTICE FOR PREVENTION OF MIGRAINE DURING THE PILL-FREE INTERVAL

If oestrogen 'withdrawal' is the mechanism for migraine arising in the pill-free interval, minimising the decline in oestrogen during the pill-free interval should prevent attacks.

Such attacks may be resolved by altering the ratio of oestrogen to progestogen or, more commonly, by bicycling or tricycling the pill (taking two or three packets consecutively before breaking for a pill-free interval) reducing the number of pill-free intervals, and thus the number of migraine attacks, from 13 to five per year. (Whitty *et al.*, 1966) Continuous COC use is an option used increasingly and is licensed in some countries, although unscheduled bleeding can be a problem. (Edelman *et al.*, 2006)

However, some women prefer to have a regular 'period'. Using natural oestrogen such as oestradiol during the pill-free interval could, if the oestrogen withdrawal theory is correct, prevent attacks of migraine at this stage of the pill cycle whilst allowing women to experience a withdrawal bleed.

3.4.2. PAPER VIII: PREVENTION OF MIGRAINE DURING THE PILL FREE INTERVAL OF COMBINED ORAL CONTRACEPTIVES USING OESTRADIOL SUPPLEMENTS

OBJECTIVES

The aim of this study was to determine whether the use of natural oestrogen supplements used during the pill-free interval could reduce the occurrence of migraine headache and associated symptoms.

METHODS

The East London and City Research Ethics Committee approved the protocol. Women who fulfilled the entry criteria were invited to participate in a double-blind placebo-controlled randomised crossover study in which each woman was given oestrogen patches (Evorel™ 50µg) or matching placebo, each to be used during the pill-free interval of 4 pill cycles allocated at random (2 cycles using oestrogen, 2 using placebo). This enabled a within-woman analysis of the data to be possible.

Most patients were recruited from outpatient clinics at the City of London Migraine Clinic and at St Bartholomew's Hospital Sexual Health Clinic. A couple of patients were also recruited following advertisements placed in the waiting room at the Margaret Pyke Family Planning Centre and in other local family planning clinics. As recruitment was poor, advertisements were also placed in local newspapers but, despite a good response, few suitable subjects were identified. All women were reviewed at the City of London Migraine Clinic before inclusion in the study. In order to fulfil the entry criteria, women had to be aged over 18 years and suffering from recurrent attacks of migraine without aura during the pill-free interval. They had had migraine without aura for at least one year, had been using the same COCs for at least six months prior to entering the study, and had no contraindications to their continuing use of COCs. All women entered gave informed consent and were free to withdraw at any stage of the study for any reason.

A full medical and headache history was taken from all women, who were then given a neurological and general physical examination. All information was recorded on the case report forms. Women were then randomly allocated to receive oestrogen or placebo patches that were to be used during the pill-free interval. Two patches were provided for each cycle: the first was to be used on the evening of the last day of the pill cycle, replaced with the second patch on the morning of the fourth day of the pill-free interval, and removed on the first day of the next pill cycle. Diary cards were provided which highlighted the correct time for patch changes. Women were instructed to record any headache or migraine occurring at any time during the four month study on diary cards, using a verbal rating scale for severity. Associated symptoms of nausea, vomiting and photophobia were also recorded, as was use of medication for acute treatment. Women were seen at the City of London Migraine Clinic after the first 2 cycles to check blood pressure and to monitor adverse events, and at the end of the 4th cycle for a third and final assessment. Trial medication was issued for two cycles at the first and second visits.

The following outcomes were assessed during the two pill-free intervals for each treatment and placebo regime:

- The number of pill-free intervals (0,1 or 2) during which at least one migraine occurred;
- The number of days during which a migraine occurred (0 to 14) over the two weeks;
- The number of migraines that were considered to be mild, moderate or severe;
- The number of days of migraine (0 to 14) over two weeks that were accompanied by nausea;
- The number of days of migraine (0 to 14) over two weeks that were accompanied by vomiting;
- The number of days of migraine (0 to 14) over two weeks that were accompanied by photophobia.

Sample size calculation and statistical methods

A crossover trial of 20 women would have over 90% power to show a difference of 50% reduction in the outcomes assessed.

Non-parametric tests (Wilcoxon one-sample test) were used to compare the median within-person differences and t-tests for comparing the mean.

RESULTS

We were unable to meet our recruitment target of 20 patients. Fourteen women, median age 33 years (range 24-42), with a median onset of migraine without aura at age 19 years (range 7 to 41) were recruited over a two-year period. The type of COC taken by each woman is listed in Table 13. Eight women took 3rd generation COCs (all monophasic) and six took 2nd generation COCs (4 monophasic and 2 triphasic).

Patient No:	COC type
1	EE 30µg / gestodene 75µg
2	EE 35µg / cyproterone acetate 2mg
3	EE 20µg / desogestrel 150µg
4	EE 35µg / norgestimate 250µg
5	EE 35µg / norethisterone 0.5-1mg
6	EE 30-40µg / levonorgestrel 50-125µg
7	EE 30µg / levonorgestrel 150µg
8	EE 35µg / cyproterone acetate 2mg
9	EE 20µg / desogestrel 150µg
10	EE 30µg / desogestrel 150µg
11	EE 30µg / levonorgestrel 150µg
12	EE 30µg / levonorgestrel 150µg
13	EE 20µg / desogestrel 150µg
14	EE 30µg / levonorgestrel 150µg

EE = ethinylloestradiol

TABLE 13: TYPE OF COC USED BY EACH PATIENT

Thirteen women had taken their current COC for at least six months, and one woman (Patient no. 13) had taken her COC for three months.

Attacks of migraine were typically reported as starting on the third day of the pill-free interval (Figure 37)

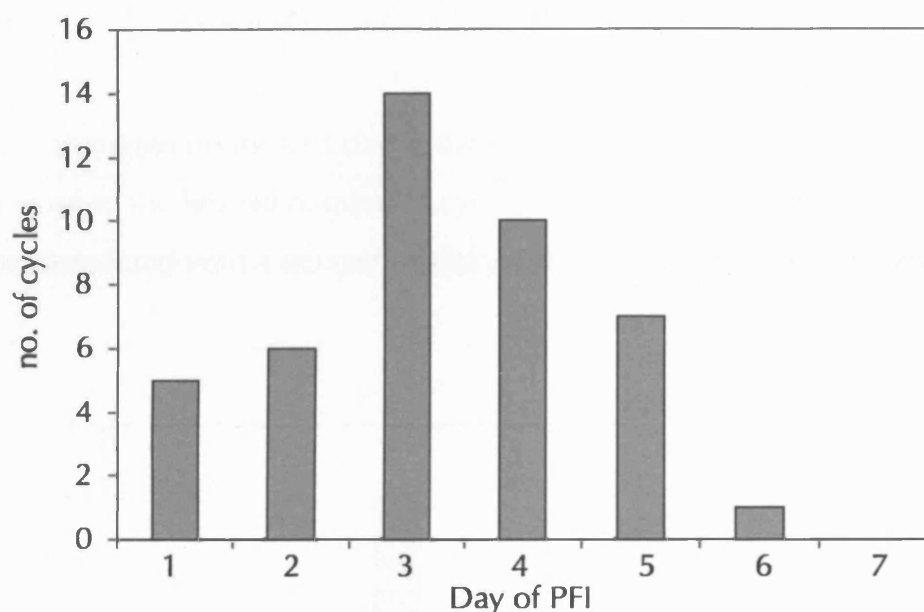


FIGURE 37: FIRST DAY OF HEADACHE DURING THE PILL-FREE INTERVAL (PFI) IN 50 CYCLES

Twelve women completed the study with full data for analysis. One woman withdrew for lack of effect after treating two cycles (one with oestrogen one with placebo) and another withdrew consent before treating.

Only two adverse events were reported that could be attributable to the study medication. Patient no. 6, who was taking a triphasic COC, reported no withdrawal bleed and no headaches or migraine in the two pill-free intervals using oestrogen patches. In day 5 of the each of the pill-free interval in which she used placebo, she experienced a withdrawal bleed and an associated migraine attack.

Patient no. 14, who was taking a monophasic COC, experienced withdrawal bleeds starting on day 4 of the pill-free interval for both placebo cycles and on day 3 and day 4 of the pill-free interval in the two oestrogen cycles. She reported that the withdrawal bleed was much lighter than usual during the two oestrogen cycles.

The only other adverse event was reported by patient no. 5 who was taking a triphasic COC and who experienced early bleeding on day 19 or 20 of the pill cycle during every cycle in the study. This event was predated participation in the study.

The effect oestrogen on the first day of the withdrawal bleed was analysed. Bearing in mind the limited number of cycles, there was a tendency for oestrogen use to be associated with a delayed withdrawal bleed (Figure 38 and Figure 39).

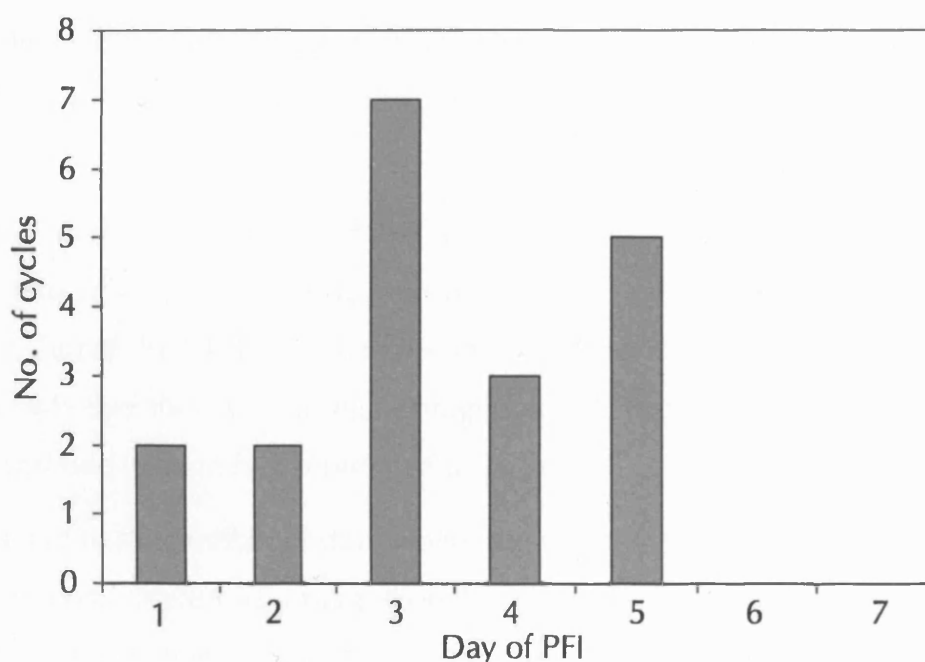


FIGURE 38: FIRST DAY OF WITHDRAWAL BLEED IN THE PILL FREE INTERVAL (PFI) IN WOMEN USING PLACEBO

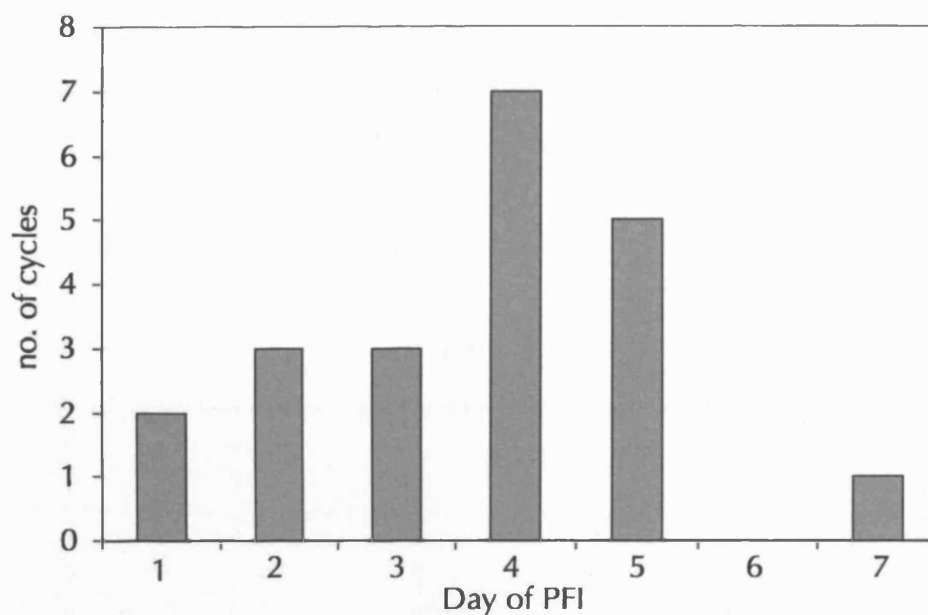


FIGURE 39: FIRST DAY OF WITHDRAWAL BLEED IN THE PILL FREE INTERVAL (PFI) IN WOMEN USING OESTROGEN

There was no evidence to suggest that women had fewer migraine attacks during pill-free intervals after using 50µg oestrogen patches compared to placebo ($P=0.55$). Ten of the 13 women had the same number of pill-free intervals with migraine whether they were using oestrogen or not, two women had fewer weeks, and one woman had more weeks (Table 14).

With respect to the number of days where the women had migraine during the pill-free interval, there was a suggestion that when the women were using 50µg oestrogen patches, they had fewer days of migraine than when they were using placebo (median difference -0.5 days [95% CI -2.0 to 1.5]), but the results were not statistically significantly different ($P=0.55$) (Table 14).

Patient no.	No. of pill free weeks (0-2)			No. of migraines (0-14)		
	E2	P	Difference	E2	P	Difference
1	2	2	0	3	4	-1
2	1	1	0	1	1	0
3	1	2	-1	3	5	-2
4	2	2	0	6	9	-3
5	2	2	0	6	11	-5
6	5	2	-2	1	3	-3
7	1	1	0	2	1	0
8	2	2	0	4	5	1
9	2	2	0	5	5	0
10	2	1	1	10	3	7
11	1	1	0	2	1	1
12	2	2	0	11	8	3
13	2	2	0	2	2	0
Median	-	-	0	-	-	-0.5

E2: oestrogen patches, P: placebo patches, Difference: E2 minus P

TABLE 14: NO. OF PILL FREE WEEKS WHEN MIGRAINE OCCURRED AND NO. OF MIGRAINES DURING EACH 2-WEEK PERIOD FOR TREATMENT AND PLACEBO

The frequencies of mild, moderate or severe migraines in women using 50µg oestrogen patches or placebo are shown in Table 15. The overall severity of migraine was assessed using a weighted difference score which suggested that women using oestrogen tended to have less severe migraine but the difference was not statistically significantly different (mean score -1.0 $P=0.67$, median score -1.0 $P=0.53$).

Patient no.	No. of migraines which were:									Weighted difference score
	Mild			Moderate			Severe			
	E2	P	Difference	E2	P	Difference	E2	P	Difference	
1	1	3	-2	1	1	0	1	0	1	1
2	1	1	0	0	0	0	0	0	0	0
3	2	4	-2	1	1	0	0	0	0	-2
4	0	0	0	1	1	0	5	8	-3	-9
5	3	2	1	1	3	-2	2	6	-4	-15
6	0	1	-1	0	0	0	0	2	-2	-7
7	1	0	1	0	0	0	0	1	-1	-2
8	1	0	1	2	4	-2	1	1	0	-3
9	2	2	0	3	2	1	0	1	-1	-1
10	4	3	1	1	0	1	5	0	5	18
11	0	0	0	0	1	-1	2	0	2	4
12	5	2	3	6	6	0	0	0	0	3
13	0	1	-1	2	1	1	0	0	0	1
Median	-	-	0	-	-	0	-	-	0	-1

E2: oestrogen patches, P: placebo patches, Difference: E2 minus P

A weighted difference score for each woman was obtained by multiplying the difference in the number of mild migraines with a score of 1, moderate migraines multiplied by 2 and severe migraines multiplied by 3; the total score for each woman is the sum.

TABLE 15: NO. OF DAYS (0 TO 14) WHEN SEVERITY OF MIGRAINE WAS MILD, MODERATE OR SEVERE; WEIGHTED DIFFERENCE SCORE IS ALSO SHOWN

There was a suggestion that women using 50µg oestrogen patches experienced more nausea with migraine than when using placebo (median difference 0.5 days [95% CI 0 to 1.0]) but the difference was not statistically significant ($P=0.35$) (Table 16). If the analysis excluded women who did not report any nausea (5 women), then the median difference was 1.0 day (95% CI -1 to 2.5), $P=0.34$.

Only two women experienced vomiting either using 50µg oestrogen supplements or placebo (1 woman had two more episodes with oestrogen, one had two less episodes) so no further comment could be made on this (Table 16).

There was no evidence to suggest that presence of photophobia was affected by oestrogen use (median difference 0, $P=0.89$) (Table 16).

Patient no.	No. of nausea episodes			No. of vomiting episodes			No. of photophobic episodes		
	E2	P	Difference	E2	P	Difference	E2	P	Difference
1	1	0	1	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	1	1	0	0	0	0	0	0	0
5	2	1	1	0	0	0	5	8	-3
6	0	3	-3	0	2	-2	0	2	-2
7	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0
9	2	1	1	0	0	0	4	5	-1
10	3	0	3	2	0	2	4	0	4
11	0	0	0	0	0	0	1	1	0
12	6	4	2	0	0	0	0	0	0
13	1	1	0	0	0	0	1	0	1
Median	-	-	0	-	-	0	-	-	0

E2: oestrogen patches, P: placebo patches, Difference: E2 minus P

TABLE 16: NO. OF DAYS (0 TO 14) WHEN MIGRAINE WAS ACCOMPANIED BY NAUSEA, VOMITING OR DISLIKE OF LIGHT

DISCUSSION

The results of this pilot study suggest that use of 50µg oestradiol patches during the pill-free interval of combined oral contraceptives shows a trend towards reducing the frequency and severity of migraine at that time. This result was not as good as expected. We had originally aimed for 20 eligible women to participate in the trial but only 14 were recruited and only 12 completed the study with full data for analysis. We observed less than a 50% difference in the outcomes assessed and, together with the smaller than intended sample size, this could explain the lack of statistical significance in our results.

The City of London Migraine Clinic is a research centre so all staff were experienced in undertaking clinical trials and diagnosing migraine. However, one of the major problems with the study was recruitment of patients, despite a long recruitment period. In clinical practice, migraine and headache occurring in the pill-free interval is common. Although many patients were identified with migraine in the pill-free interval, few were prepared to enter into a formal clinical trial and usually requested treatment rather than trial medication. This was particularly the case with younger women and it is notable that the median age of patients entering this study was 33 years – older than the usual population of pill users.

Transdermal patches were used as these provide stable plasma levels of oestradiol.(Kuhl, 1990) In hindsight, the 50µg dose of oestradiol was too low and a 100µg dose should have been used. Several women in the present study went on to use 100µg oestradiol patches during the pill-free interval with success. A better trial design would have been to use combinations of placebo and 50µg oestradiol patches to achieve pill-free interval supplements of placebo, 50µg patches and 100µg patches, each for two cycles. This would have extended the trial by a further two cycles, which might potentially have been a problem for recruitment. Against this concern, the main problem with recruitment was getting women to participate in the first instance; once they were in the trial, continuance was good. Ideally, a 150µg comparative dose would have been useful but would have raised issues of the acceptability of using three patches simultaneously.

Alternative strategies to consider include oral oestrogens during the pill-free interval or extended regimes of pill taking in women diagnosed with migraine during the pill-free interval. Although trial data for efficacy in headache exists, placebo controlled trials in women with migraine are lacking.(Calhoun, 2004; LaGuardia *et al.*, 2005; Sulak *et al.*, 2007)

Trials designed formally to assess the comparative frequency of migraine in women taking COCs containing different progestogens would also be valuable as the results could aid the decision about the choice of COC for women with migraine without aura.

Studies to identify other potential mechanisms of migraine in the pill-free interval are also recommended, as oestrogen withdrawal may be only one of several differing mechanisms.

SUMMARY OF FINDINGS

There was a non-significant trend for women using 50µg oestrogen supplements in the pill-free interval to have fewer migraines and for attacks to be less severe compared to women using placebo.

3.5. CONCLUSIONS FROM PART THREE

If the hypothesis that menstrual migraine is associated with oestrogen 'withdrawal' is correct, it follows that menstrual migraine should be prevented with exogenous oestrogen supplements to inhibit the endogenous late luteal drop in oestrogen.

From the data presented above, the following points can be concluded:

- The optimal dose for perimenstrual prophylaxis is 100µg oestradiol patches or 1.5mg oestradiol gel.
- In order to prevent the late luteal drop in oestrogen, the optimal timing for perimenstrual prophylaxis appeared to be from the luteal phase oestrogen peak (day -6), continuing through to the early follicular rise of endogenous oestrogen (day +2).
- To time perimenstrual prophylaxis effectively, it is necessary to predict the onset of menstruation.
- Although the median length of the menstrual cycle was 28 days in the women participating in our trials, there was a wide inter- and intra-individual range in cycle length, making it difficult to predict menstruation.
- Identification of ovulation using a fertility monitor enabled prediction of menstruation and accurate timing of oestrogen supplements, irrespective of cycle length.
- Perimenstrual oestradiol supplements inhibited the late luteal drop in oestrogen and prevented menstrual attacks in some, but not all, women.
- The benefits of treatment were offset by a delayed oestrogen 'withdrawal' migraine in some women.
- There was a trend to suggest that migraine in the pill-free week of combined oral contraceptives could be prevented with oestradiol supplements although, based on the dose of oestrogen and the number of participants, the results were not significant. It is likely that the 50µg dose is too low for optimal clinical benefit.

These findings provide limited support for the hypothesis that menstrual migraine can be prevented using oestradiol supplements. Reasons for the lack of clinical efficacy include failure to achieve adequate stable levels of oestradiol, too short a duration of prophylaxis so that supplements were stopped before the follicular rise in endogenous oestrogen, and suppression of endogenous oestrogen. Further, the study included women with menstrually-related migraine, in whom migraine is only partially hormone dependent.

Additional findings were that oestrogen 'withdrawal' migraine is independent of menstrual bleeding and is independent of other menstrual cycle hormones. Given that migraine can occur when oestrogen is 'withdrawn' during the hormone free interval of combined hormonal contraceptives, oestrogen 'withdrawal' migraine is also independent of ovulation.

PART 4:
SUMMARY OF RESEARCH

The results of the research presented in this thesis support the initial hypotheses:

1. Menstrual migraine is a discrete clinical entity

- 1.1. Migraine in menstruating women is most likely to occur on or between two days before the start of menstruation and the first three days of bleeding.
 - 1.1.1. Pure menstrual migraine can be defined as 'attacks of migraine without aura occurring exclusively on or between days -2 to +3 of menstruation in at least two out of three menstrual cycles and at no other times of the cycle'.
 - 1.1.2. Menstrually-related migraine can be defined as 'attacks of migraine without aura occurring on or between days -2 to +3 of menstruation in at least two out of three menstrual cycles with additional attacks of migraine with or without aura at other times of the cycle'.
- 1.2. Compared to non-menstrual attacks, menstrual attacks of migraine are significantly more severe and associated with nausea and vomiting, are longer, are less responsive to treatment, and are more prone to relapse.

2. Menstrual migraine is associated with oestrogen 'withdrawal'

- 2.1. In women with menstrual or menstrually-related migraine incidence was inversely related to rising and falling phases of oestrogen across the menstrual cycle.
 - 2.1.1. Compared to the expected incidence, the highest incidence of migraine was during the late luteal/early follicular phase of falling oestrogen.
 - 2.1.2. Compared to the expected incidence, the lowest incidence of migraine was during the follicular phase of rising oestrogen and the luteal phase of rising oestrogen.

- 2.2. There is circumstantial evidence that migraine during the hormone-free interval of combined hormonal contraceptives is associated with oestrogen 'withdrawal'.

3. Menstrual migraine can be prevented with oestradiol supplements

- 3.1. In women with menstrual migraine not using exogenous hormones:
 - 3.1.1. To maintain mid-luteal oestradiol levels, the optimal dose for perimenstrual prophylaxis is at least 100µg oestradiol patches or 1.5mg oestradiol gel.
 - 3.1.2. Based on the timing of the luteal oestradiol peak and the follicular phase oestradiol nadir, the optimum timing for gel use was considered to be from 6 days before menstruation to the second full day of bleeding.
- 3.2. Identifying ovulation enabled accurate prediction of menstruation and hence accurate timing of the start of gel use.
- 3.3. In women with menstrual migraine who are not using exogenous hormones:
 - 3.3.1. 1.5mg oestradiol gel used for eight days perimenstrually significantly reduced the frequency and severity of migraine during treatment compared to placebo.
 - 3.3.2. Oestradiol treatment was followed by an increase in migraine associated with delayed oestrogen 'withdrawal', the highest incidence on the third day following the last day of treatment.
- 3.4. In women with menstrual migraine taking combined oral contraceptives:
 - 3.4.1. The highest incidence of migraine was on the third day during the pill-free week.
 - 3.4.2. Use of 50µg oestradiol supplements during the pill-free week resulted in fewer and less severe migraine attacks compared to placebo but the difference was not significant.

PART 5:
OPPORTUNITIES FOR FUTURE RESEARCH

This thesis marks a waypoint in my research into the association between oestrogen and migraine being as much a starting point for future work as a summary of research to date. Along this journey I have sought to develop a definition for menstrual migraine in order to further research into the relevant mechanisms. Such delineation has enabled studies to confirm the association between migraine without aura and falling levels of oestrogen in the late luteal phase of the menstrual cycle. Notably, although I set out to find mechanisms for menstrual migraine, it is apparent that menstruation is only an association since oestrogen ‘withdrawal’ migraine can occur in the absence of menstrual bleeding and, indeed, in the absence of ovulation.

Although there was a clear inverse association between migraine incidence and oestrogen levels across the menstrual cycle, the results of supplementing oestrogen at the expected time of oestrogen ‘withdrawal’ were disappointing. Strategies to reduce post-dosing oestrogen ‘withdrawal’ need to be studied. Clinically, starting oestrogen supplements in the late luteal phase and tapering the dose through the early follicular phase seems effective but a placebo-controlled study is necessary to both confirm this impression and to assess the effect on follicular phase endogenous oestrogen.

Given the contraceptive needs of many women with migraine, further research into the effects of hormonal contraception is warranted. There are no studies assessing anovulatory progestogens such as the intramuscular depot medroxyprogesterone acetate (DMPA), subdermal etonogestrel and oral desogestrel. Subdermal etonogestrel and oral desogestrel inhibit the LH surge, but do not inhibit FSH.(Croxatto & Makarainen, 1998) Consequently, these methods are associated with fluctuating follicular phase oestrogen levels, which could act as a migraine trigger.(Somerville & Carey, 1970) Even with DMPA which, like combined hormonal contraceptives inhibits both FSH and LH, migraine is reported in association with breakthrough bleeding. The mechanisms for this are unknown although improvement is noted in women achieving amenorrhoea.(Somerville & Carey, 1970)

Why are some women with migraine susceptible to oestrogen ‘withdrawal’ and other women are not? The results presented in this thesis suggest that susceptible

women may have high luteal phase oestrogen levels, compared to women who do not experience menstrual attacks. This might explain why menstrual migraine is more prevalent in the perimenopause, since some women experience high fluctuating oestrogen levels.

Future studies should confirm or refute the proposed definition of 'menstrual' migraine, perhaps by analysing the peak incidence of migraine during the menstrual cycle in a larger number of migraineurs. Prospective records of migraine and menstrual periods would need to be recorded in different age groups of women who were not on any form of hormonal treatment or prophylactic migraine treatment. The duration of records should be for a minimum of three cycles. Longer records would be preferable but many women may not maintain accurate records over lengthy study periods. If sufficient data are collected it would be possible to analyse other subgroups, e.g. women with exclusively premenstrual attacks or those with attacks exclusively occurring at the time of the menstrual flow, to assess differences. Further, the type of migraine and duration of attacks in 'menstrual' migraineurs could be examined.

Are attacks of 'menstrual' migraine always without aura? Women who have both types of migraine are more likely to have migraine *without* aura associated with oestrogen 'withdrawal' and attacks *with* aura at other times of the cycle. Research not presented in this thesis suggests that migraine *with* aura occurs at times of high oestrogen levels, such as when taking combined hormonal contraceptives or hormone replacement therapy, and during pregnancy. (MacGregor & Guillebaud, 1998; MacGregor, 1999a; MacGregor, 1999b; MacGregor, 2004, 2007a, b)

There is a need for further evidence to support or refute the concept of 'menstrual' migraine as a specific nosological entity. To date, most of the studies cited have included small numbers of women over few cycles and have combined data from women using hormonal treatments with those who are not. Further, since the issue is whether or not migraine at menstruation is different from non-menstrual attacks within the same woman, studies would be more relevant if they undertook within-woman analyses. In order better to understand the pathophysiology and to provide effective treatment, it is important to assess the association prospectively

over a large number of cycles in a large number of women who have an accurate diagnosis of migraine.

In addition, there is the issue of whether or not migraine associated with withdrawal bleeding during the pill-free interval of combined oral contraceptives or with progestogen-withdrawal bleeding in women using hormone-replacement therapy constitutes 'menstrual' migraine. Since migraine associated with withdrawal bleeding resulting from hormonal contraception or hormone replacement therapy may have a different pathophysiology from menstrual migraine resulting from the hypothalamic-pituitary-ovarian cycle, these groups should be studied separately.

Studies should establish if there is a continuing relationship between migraine attacks and menstruation over a longer period of time. Most women have difficulty in remembering accurately the effects of hormonal events on their headaches and interview bias can affect the results of retrospective studies. It is therefore important that all evidence should be from prospective studies. Some women do not initially relate their attacks to menstruation but only notice a link in the perimenopause or after some specific hormonal event e.g. pregnancy or use of oral contraception. This may be due to subjective bias but could represent a true change in pathophysiology. An ideal study would be to follow women over many years and assess the changes in the timing and type of migraine attacks relating to known hormonal changes. An example is the temporal relationship of migraine during pregnancy and post partum. The effects of hormonal events on non-migraine headaches also merit further study.

Then there is the question of whether the effect of oestrogen is primary or secondary. Regulation of the menstrual cycle is complex with ovarian steroids playing a limited role in the overall control. It is likely that the chemical alteration more directly responsible for migraine is the effect of the changing hormonal environment on other biochemical and metabolic pathways, rather than a direct effect of sex hormones.

Ovarian steroids cross the blood-brain by passive diffusion, with brain levels mirroring blood levels.(Aloisi, 2003) There is evidence that ovarian steroids can be produced within the central nervous system.(Stoffel-Wagner, 1993) As

neurosteroids, oestrogen and progesterone can influence the pain processing networks and vascular endothelium involved in the pathophysiology of migraine. Both oestrogen and progesterone also have a clinically relevant effect on vascular tone.(Sarrel, 1999) Further, close interrelationships between oestrogens and brain neurotransmitters have been confirmed, including the catecholamines, norepinephrine, dopamine and the endorphins. Therefore, hormonal changes could trigger some alteration in activity of the hypothalamic-pituitary-adrenal axis, exposing susceptible women to a migraine attack. Accordingly, migraine has been shown to increase following gonadotropin-releasing hormone (GnRH) analogue induced hypothalamic-pituitary-ovarian axis down-regulation.(Amir *et al.*, 2005)

Fluctuating levels of oestrogen and progesterone in the luteal phase of the menstrual cycle affect levels of brain dopamine.(Magos *et al.*, 1985; Murialdo *et al.*, 1986) Oestrogen also facilitates the glutaminergic system, potentially enhancing neural excitability. In contrast, progesterone appears to activate GABAergic systems, suppressing neuronal reactivity, and modulates the effects of oestrogen on the central nervous system.(Martin & Behbehani, 2006) Oestrogen has potent effects on the serotonergic system, increasing serotonergic tone.

Serotonin-producing neurons are sensitive to the presence or absence of ovarian hormones.(McEwan, 2002) Fluctuating levels of oestrogen and progesterone in the luteal phase of the menstrual cycle affect levels of brain serotonin and abnormalities of the serotonin system in menstrual migraine has been reviewed.(Silberstein & Merriam, 1993) This association might account for efficacy of perimenstrual prophylaxis with triptans and warrants further evaluation.(Newman *et al.*, 1998; Newman *et al.*, 2001; Silberstein *et al.*, 2004; Tuchman *et al.*, 2005; Mannix *et al.*, 2007; Brandes *et al.*) To this end, we are examining the association between urinary serotonin, precursors and metabolites, oestrogen and migraine across the menstrual cycle in women with pure menstrual or menstrually-related migraine.

That oestrogens do not affect all women with migraine might be explained by the intrinsic oestrogen receptor sensitivity of the hypothalamic neurons. Limited data imply that this may have a genetic basis.(Berman *et al.*, 2006) Experimental

studies in rats suggest that abnormalities in how oestrogen modulates neuronal function in migraine may be due to a mismatch between its gene-regulation and membrane effects. A decline in oestrogen levels modulates central sensitization, increasing the pain and disability of the migraine attack.(Welch *et al.*, 2006; Martin *et al.*, 2007) We are currently undertaking further research to identify genes that may play a role in menstrual migraine.

RESEARCH IN PROGRESS

EFFECT OF MIGRAINE, THE MENSTRUAL CYCLE, AND PERIMENSTRUAL OESTRADIOL SUPPLEMENTS ON THE URINARY METABOLITES SEROTONIN (5-HT), 5-HYDROXYINDOLE ACETIC ACID (5-HIAA) AND TRYPTOPHAN IN WOMEN WITH MENSTRUAL OR MENSTRUALLY-RELATED MIGRAINE

Experimental and physiological studies have suggested a relationship between serotonin (5HT) and menstrual cycle hormones.(Martin & Behbehani, 2006) Given the implication of 5HT in migraine, and the benefits of perimenstrual triptan prophylaxis, this association with oestrogen may be important to our understanding of the pathophysiology of menstrual migraine. However, human data are lacking

We are undertaking a pilot study to assess urinary 5HT, 5-hydroxyindoleacetic acid (5HIAA) - the metabolite of 5HT, and tryptophan - a precursor for serotonin, across natural menstrual cycles in women with menstrual migraine. The effects of perimenstrual oestrogen supplements on urinary 5HT, 5HIAA and tryptophan will also be assessed.

The hypotheses are that:

1. Changes in urinary 5HT, 5HIAA and tryptophan parallel changes in urinary oestrogen metabolites.
2. Urinary 5HT, 5HIAA and tryptophan increase in direct temporal relationship to migraine attacks.

Urine samples from Studies V, VI, and VII were frozen at -20C and are available for further analysis. Samples from five women, randomly selected from the study of 38 women who completed the oestrogen and menstrual migraine studies, will be analysed for serotonin and 5HIAA. For each woman, daily samples from the three placebo and three oestrogen treated cycles will be analysed. Approximately 180 samples will be analysed for each woman, i.e. approximately 900 samples in total.

For the first hypothesis, data will be analysed separately for each woman and pooled with respect to urinary 5HT, 5HIAA and tryptophan levels. These results, across each day of the menstrual cycle, will be compared with existing data on

oestrogen levels from the same samples to assess any correlation between urinary oestrogen and urinary 5HT, 5HTIAA and tryptophan across treated and untreated cycles.

For the second hypothesis, urinary 5HT, 5HIAA and tryptophan levels for each woman will be compared to diary data, previously collected, regarding the timing of migraine attacks to assess any migraine specific changes in serotonin metabolism.

Depending on the outcome of this initial study, we may extend the study to analyse the samples from the remaining 33 women who participated in the original studies.

THE GENETICS OF MENSTRUAL MIGRAINE

The ovarian hormones play a complex role in the central nervous system, and their effects appear to be mediated by both genomic and non-genomic mechanisms.(Kelly *et al.*, 1977; Gordon & Diamond, 1981; Biegon *et al.*, 1983; Nabekura *et al.*, 1986; Bethea, 1993)

Animal studies support interactions with various neurotransmitter systems, as well as a role in neuronal excitability. Further, oestrogens can affect vascular tone, most likely through mechanisms involving nitric oxide release.(Caulin-Glaser *et al.*, 1997; Florian *et al.*, 2004) Thus, genes involved in hormonal pathways represent likely candidates for migraine susceptibility.

Griffiths and co-workers at the Genomics Research Centre, Australia, have been investigating the possibility that genes involved in hormonal pathways may play a role in migraine susceptibility.(Colson *et al.*, 2006; Colson *et al.*, 2007) In 2004 they investigated the role of the oestrogen receptor 1 (ESR1) gene in migraine by analysing the G594A SNP in 575 migraineurs and 575 controls (analysed as two independent study groups). Results of both studies showed a significant association of the A allele of the SNP with migraine.(Colson *et al.*, 2004)

More recently, they investigated the progesterone receptor (PGR) PROGINS insert in the same two study groups.(Colson *et al.*, 2005) Results also implicated a role for this gene in migraine in both independent study groups. They found that women who carried a copy of both PR and ESR1 risk alleles were 3.2 times more likely to suffer from migraine, an effect that is greater than the independent effects of these genetic variants on disease susceptibility. It is anticipated that this association will be stronger in women with menstrual migraine, who have a strong hormonal trigger for attacks.

To this end, we are undertaking a case-control directed to identifying genes that play a role in menstrual migraine (pure menstrual migraine and menstrually-related migraine), in collaboration with the Genomics Research Centre, Griffith University, Australia.

The hypothesis is that ESR and PGR INSERT, which have already been implicated with susceptibility variants in typical migraine, are likely to play a more specific role in menstrual migraine.

The primary objective is to look at the specific genes, ESR and PGR Insert, which are already implicated. The secondary objective is to look at other genes, not previously studied, which may also play a role in menstrual migraine.

The results of this research collaboration have the potential to provide valuable insights into the genetic susceptibility of menstrual migraine. If the genes that play a role in this sub-type of migraine can be identified, it should be possible to develop objective ways of testing for these genes as a diagnostic tool. Accurate diagnosis of menstrual migraine can aid the selection of currently available treatments. Further, identification of the genes involved could ultimately lead to the development of more effective treatments, targeted to the specific genes.

PART 6:
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APPENDIX 2:
FERTILITY MONITOR FEEDBACK

Summary of Questionnaire Responses n=35 (3 questionnaires not returned)

Question	Response	Number	%
Expectations Met?	Yes	15	43%
	No	8	23%
	Partially	10	28%
	Not Sure	2	6%
		35	100%
Migraine Management Influence?	Positive	26	74%
	Negative	0	0%
	Both	2	6%
	N/A	1	3%
	No Change	6	17%
		35	300%
Keeping Diaries Useful?	Yes	33	94%
	No	1	3%
	Both	1	3%
		35	100%
Using Fertility Monitor Useful?	Yes	33	94%
	No	1	3%
	Blank	1	3%
		35	100%
More Info Reg Mens Cycles?	Yes	31	89%
	No	4	11%
		35	100%
Monitor As Treatment Aid?	Yes	27	77%
	No	6	17%
	Not Sure	2	6%
		35	100%

Were your expectations about taking part in the study met?

Volunteers' expectations centred on finding out about causes of their migraines, how to prevent attacks and how to deal with them. Many were keen to see if there really was a pattern between their hormones and migraine attacks, to see if there was an alternative to their usual treatments and whether symptoms could be lessened. Some women wanted to help others in the future.

43% of respondents felt that their personal expectations had been met:

"I entered into trial with an open mind. At worst, I would at least be assisting the research. At best it might help me to find a better way to manage the migraines...It gave me a much better understanding as to why my migraines occur."

28% felt that their expectations were partially met:

"I did find out a lot about my monthly cycles but the gel didn't seem to make a lot of difference."

23% felt that their expectations were not met:

*"No conclusive results emerged about my particular hormonal pattern."
"My cycle was not as regular as before because of my age."*

How did you feel that taking part in this study influenced (positively or negatively) the management of your migraines?

Although for some volunteers (17%) there was no change in the management of their migraines, most volunteers who responded felt that taking part in the study influenced the management of their migraines in a positive way (74%), although this was for various reasons:

"It was extremely positive for me, because apart from maybe finding my 'wonder cure', I have been given some very helpful advice on how I might help reduce the impact of migraine on my life."

"Knowing that I could be doing something positive helped me mentally."

"Positive influence as I didn't feel that I was so different from other women of my age."

No one felt that there was only a negative influence on their migraine management, but two volunteers did record negative aspects:

"By keeping the diaries and using the gel I was more reluctant to try other treatments pain killers because I felt that they might mask any effects from the gel."

"Initially it was good to feel involved...later on in the study I had difficulty doing all I was required to do."

Did you find keeping diaries helpful?

Most volunteers (94%) felt that keeping migraine diaries was helpful, although one volunteer wrote "NO" because she,

"Already did this" and another felt that she,

"...was more reluctant to try other treatments."

Generally, the volunteers felt that the diaries were useful for looking at patterns and overall pictures of migraine attacks:

"Could see definite patterns."

"Helpful to give accurate picture of my migraines."

"They show frequency and timing of attacks."

Other comments included:

"Helps track causes and triggers (may be able to use this to recognise triggers in the future)."

"Learnt when to expect headaches..."

"Memory plays strange tricks; a diary is an easy reference tool and helps in discussion with your doctor etc."

Did you find using the fertility monitor helpful?

Most volunteers (94%) thought that using the fertility monitor was helpful and give a variety of reasons often relating to knowledge and the predictability of periods and therefore migraines:

"It was interesting and revelatory to realise I wasn't ovulating every month and that it made no difference to the headaches!"

"Helps me plan around my migraine attacks."

"This was useful in confirming exactly when my oestrogen level was going to start dropping."

"....helped knowing the end of cycle was on its way."

"Interested to see ovulation and changes in my body."

"Ability to pin-point when period was due."

"You could adjust your life style because you had more of an idea when migraine would occur....it helped plot more accurately [than the diaries]."

"Gave me a sense of control by knowing what was happening to my body and when."

"....reassuring to know cycles were generally normal."

"Helped provide a specific data to contribute to my more personal interpretations of what was going on in my body!"

"Most definitely! It taught me a lot about my menstrual cycle, which has never been completely reliable and regular!"

"I found I could judge the "danger period" for migraine very accurately."

"Very helpful – as cycle not 100% regular I found it very useful."

"Helped me to know when period approaching and therefore attacks."

"Interesting to see what's going on "inside" and when I am ovulating."

"Showed me how body was really working."

"Could plan ahead of an attack."

"I found just being totally tuned in to where my body was in its month cycle helped me to "manage" attacks. For instance, I felt more confident as to whether I could or could not risk a glass of wine!"

Did you feel that you have gained more information about your own menstrual cycle?

Most volunteers (89%) felt that they had definitely gained more information about their menstrual cycle:

“Yes – definitely – very interesting.”

Of those that did not gain more information (11%), this was because they were already aware of the link between their periods and migraines:

“No not really, other than confirming what I already thought that my migraines were linked to menstruation.”

Would you be willing to use the monitor as a treatment aid for you in the future?

Most volunteers (77%) felt that they would be willing to use the monitor as a treatment aid despite some having more difficulty with it than others:

"If I could be sure it would not malfunction and if the purpose was made clear for example, it would reduce the frequency and severity of my migraines. Urine samples are a nuisance though."

"To remind me IF I should be taking oestrogen gel."

"...anything to help predict the fluctuation in hormone levels. Perhaps this would help if the monitor read daily urine samples all through the month."

"If I felt this would help my situation, I would try anything!"

"Yes, if it helped pinpoint time for treatment and migraine prevention."

"This I found very easy to use it became part of my daily routine, I even used it when camping. No problem."

"Although I did find daily urine testing quite a commitment- especially away from home."

"Yes, in fact, I have missed the monitor a lot since the trial ended. Slightly irregular cycles mean I have to guess a lot more than I'd like when it comes to applying the gel."

"I feel it keeps a more accurate track of the menstrual cycle."

"Oh yes please. I would know when to take supplements, as my periods are becoming a little erratic now."

"In fact it would help me to be more clear when to commence using the oestrogen supplement each month."

"Would obviously only use it if it were for a purpose. Didn't find it time consuming and it was interesting to see fluctuations."

"Once used to it, it was not problem to use. It helped me work out when an attack was possible."

"It was invaluable at working out exactly when my period was due so I could try and take preventative measures before migraine started."

"It made me feel very 'in control'."

Two respondents (6%) were not sure whether they would use the monitor and six more (17%) felt that it would not be of benefit to them or had another reason:

"No as I did not feel it was of benefit to me regarding my migraines."

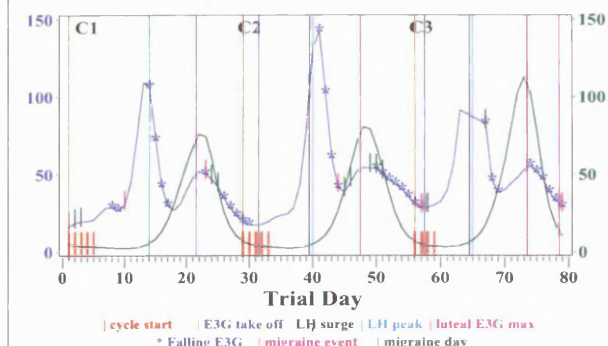
"The monitor is very easy to use, but I prefer Almogran to oestrogel."

"I found the use of the monitor restricting and I became "regimented" in my day to day living."

APPENDIX 3:
INDIVIDUAL GRAPHS OF HORMONES LEVELS FOR EACH
PATIENT (PAPER V AND PAPER VII)

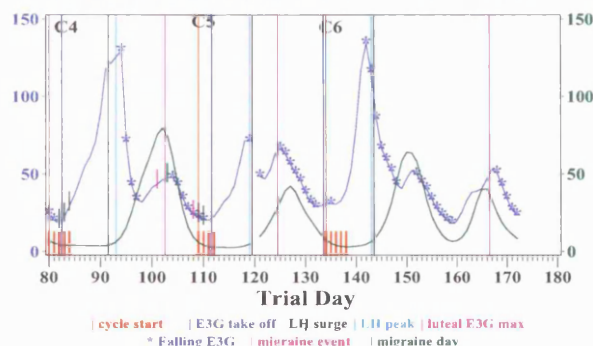
LMC pre-treatment: Vol. 02 Set 1

smoothed E3G ng/mL smoothed P3G ug/mL



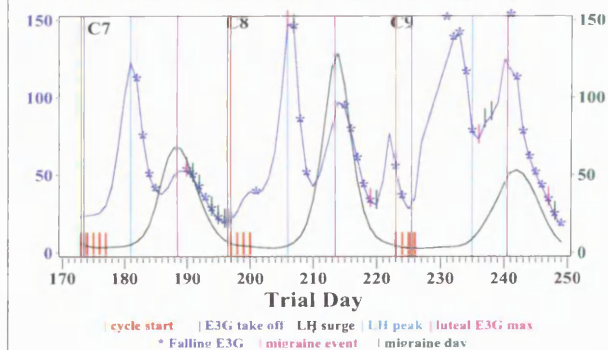
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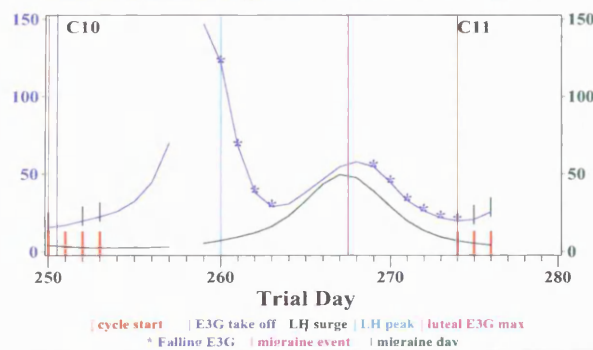
LMC pre-treatment: Vol. 02 Set 3

smoothed E3G ng/mL smoothed P3G ug/mL



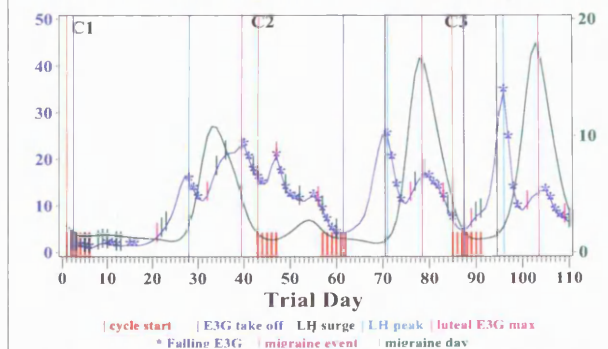
LMC pre-treatment: Vol. 02 Set 4

smoothed E3G ng/mL smoothed P3G ug/mL



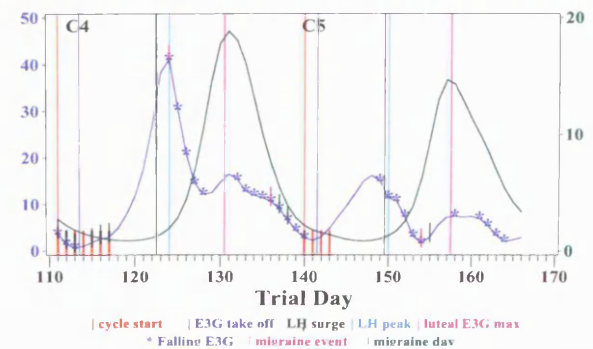
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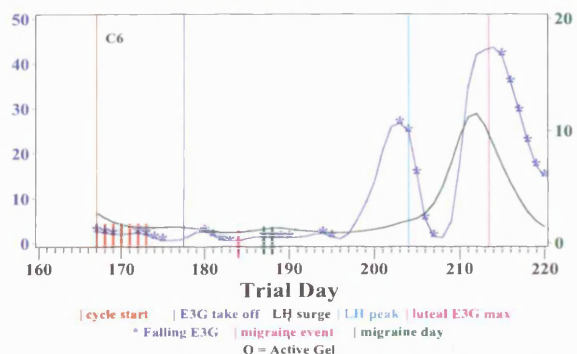
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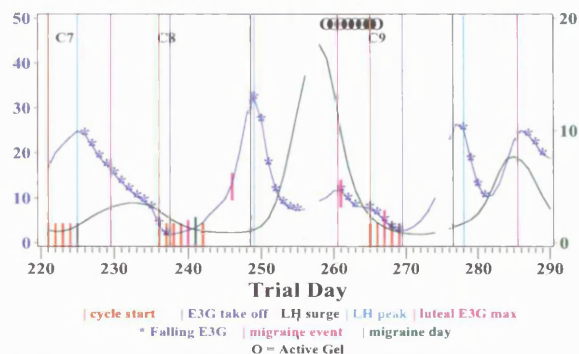


LMC post-treatment: Vol. 03 Set 2

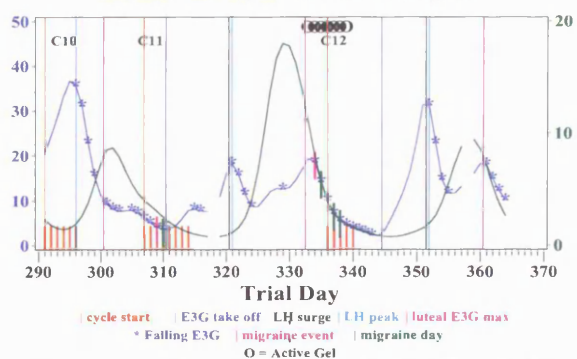
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**LMC post-treatment: Vol. 03 Set 3**

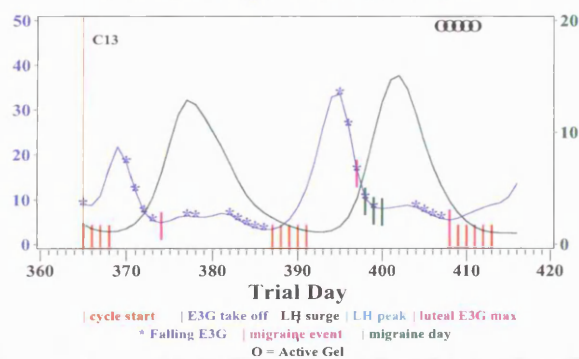
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**LMC post-treatment: Vol. 03 Set 4**

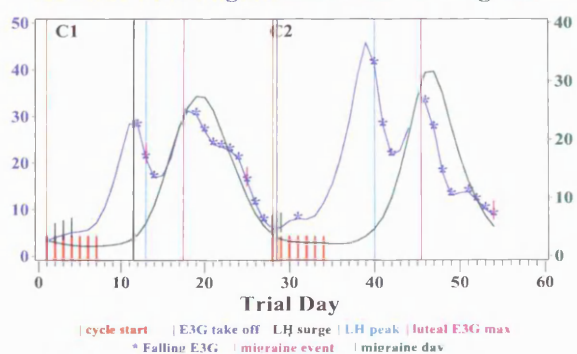
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**LMC post-treatment: Vol. 03 Set 5**

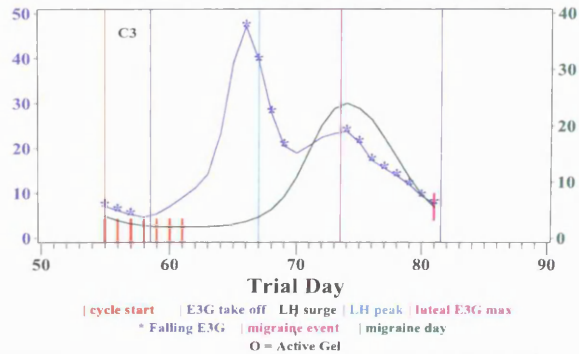
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**LMC pre-treatment: Vol. 04 Set 1**

smoothed E3G ng/mL smoothed P3G ug/mL

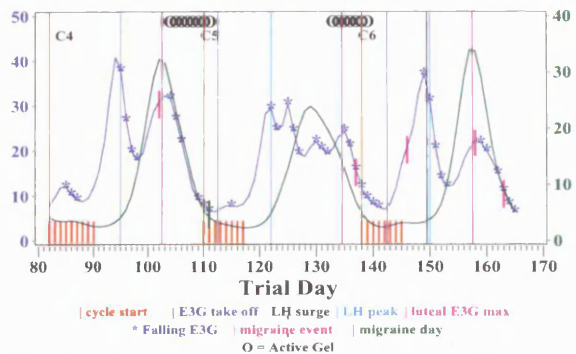
**LMC post-treatment: Vol. 04 Set 1**

smoothed E3G ng/mL smoothed P3G ug/mL

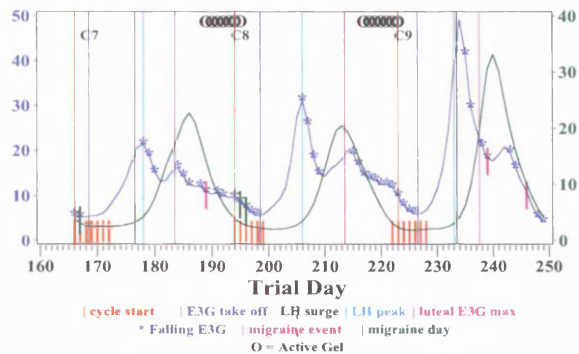


LMC post-treatment: Vol. 04 Set 2

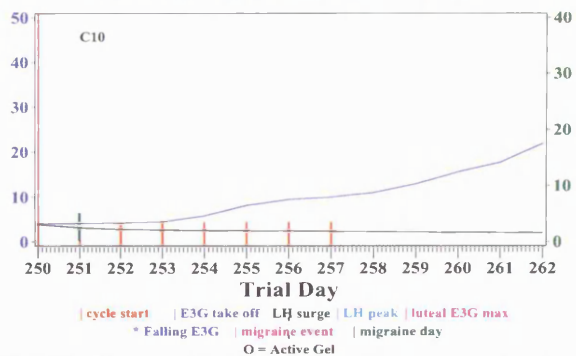
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**LMC post-treatment: Vol. 04 Set 3**

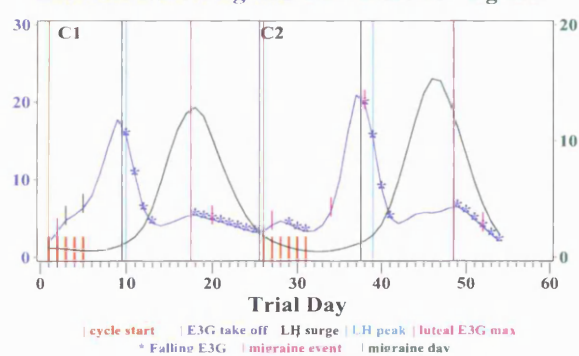
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**LMC post-treatment: Vol. 04 Set 4**

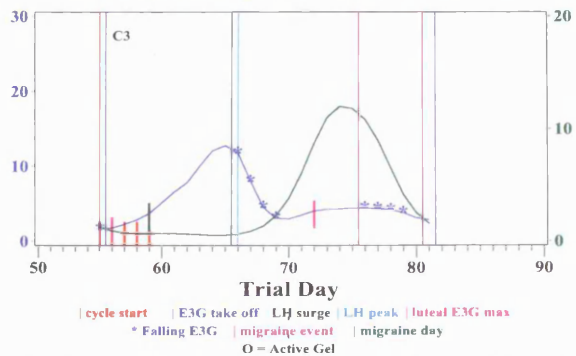
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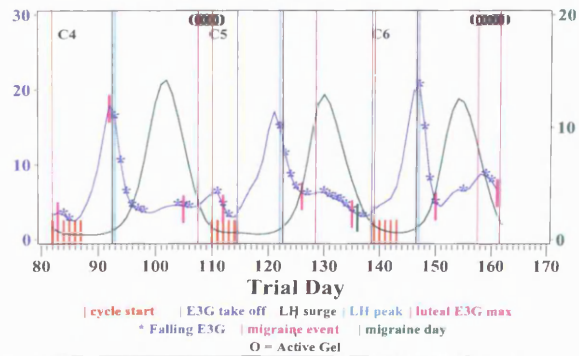
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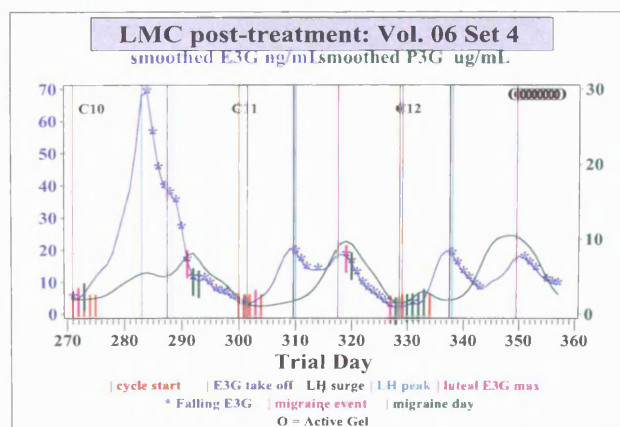
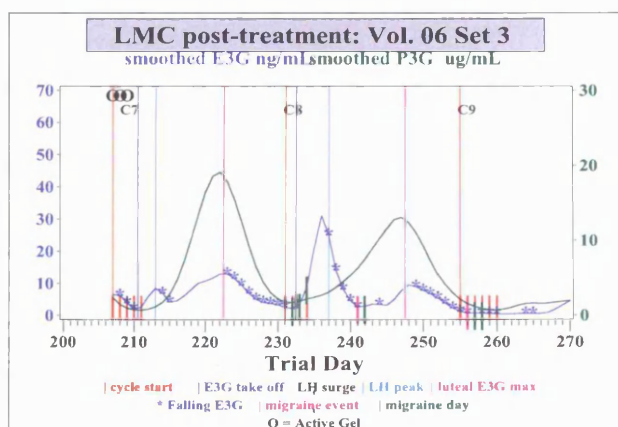
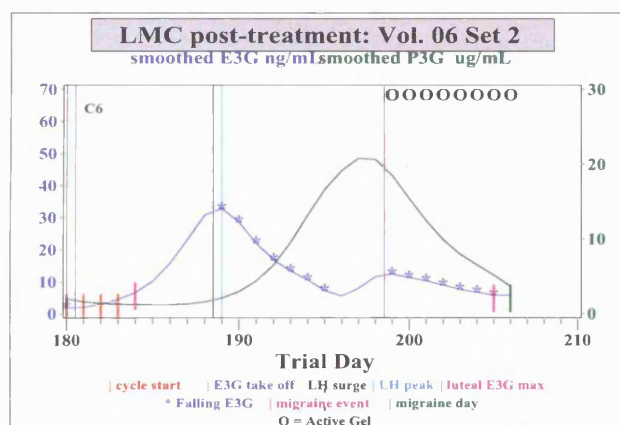
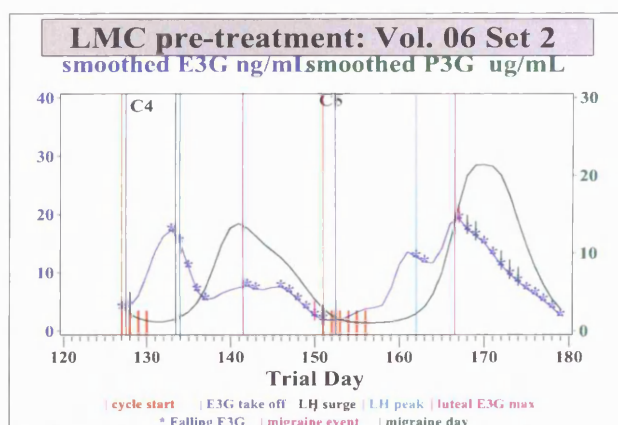
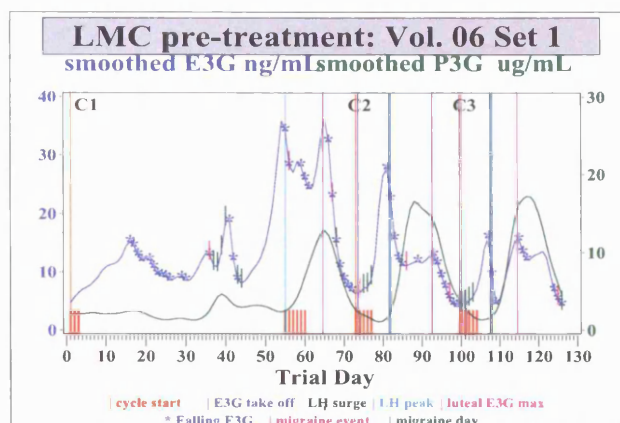
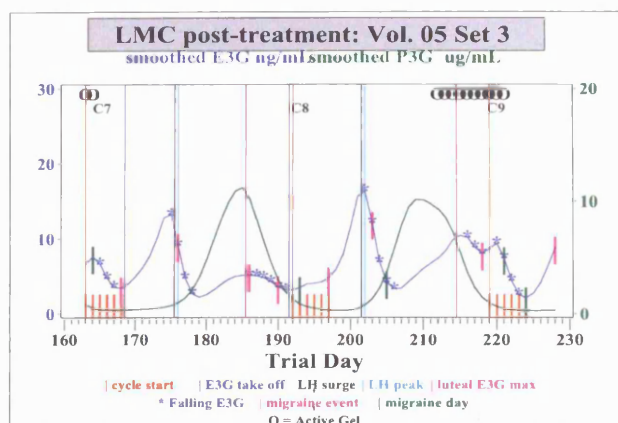
**LMC post-treatment: Vol. 05 Set 1**

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**LMC post-treatment: Vol. 05 Set 2**

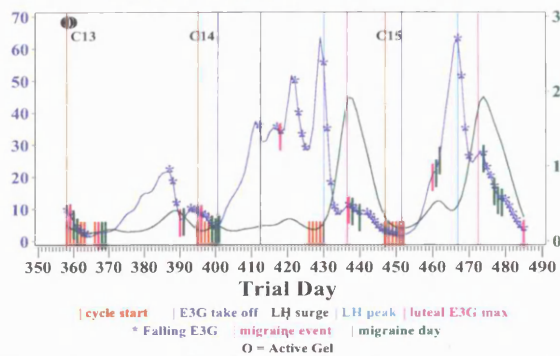
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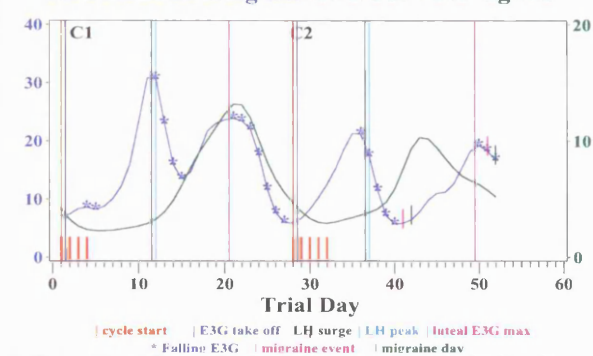


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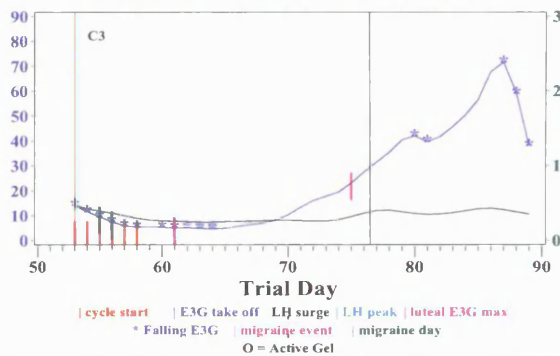
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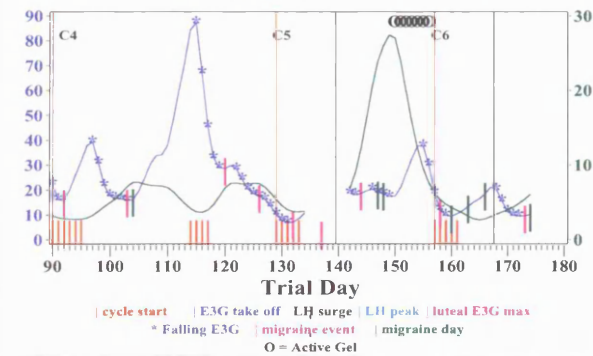
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**LMC post-treatment: Vol. 07 Set 1**

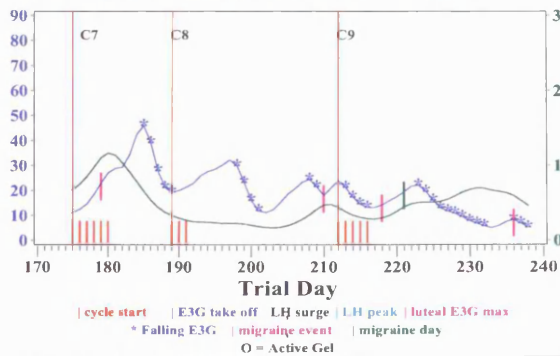
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**LMC post-treatment: Vol. 07 Set 2**

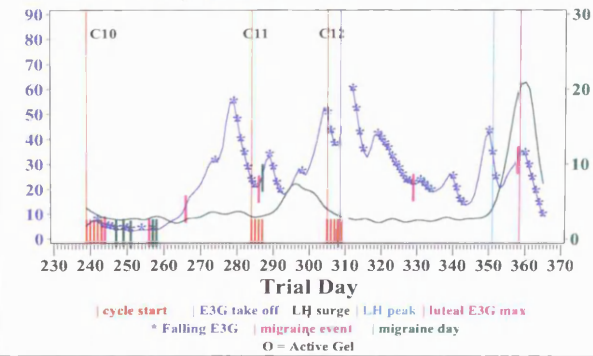
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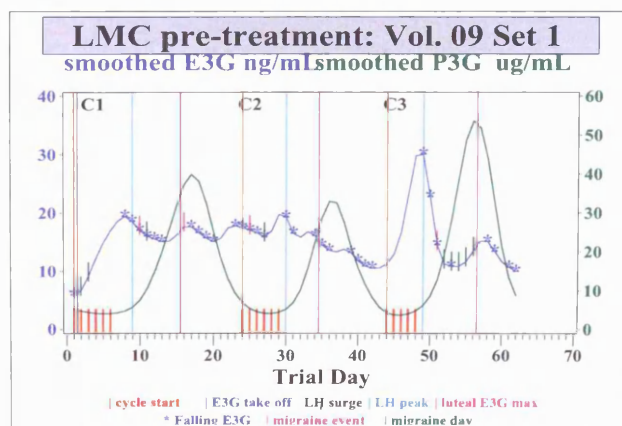
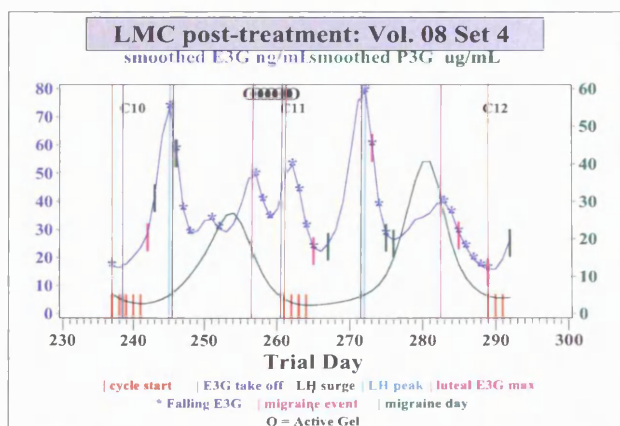
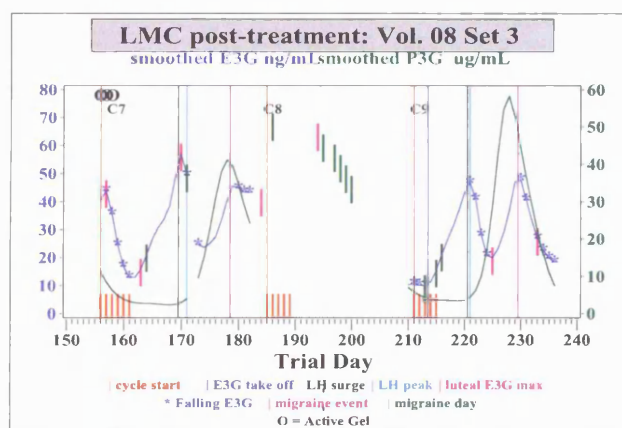
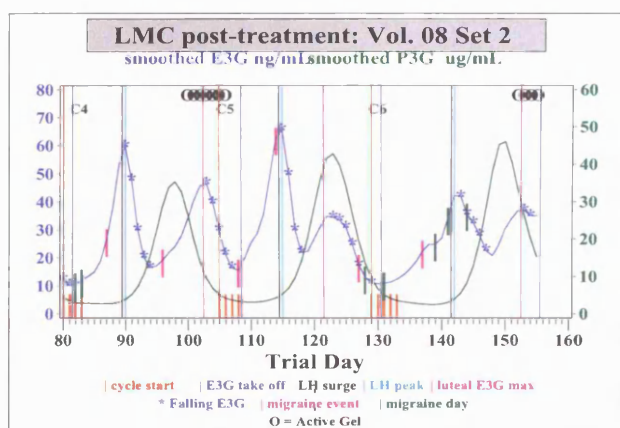
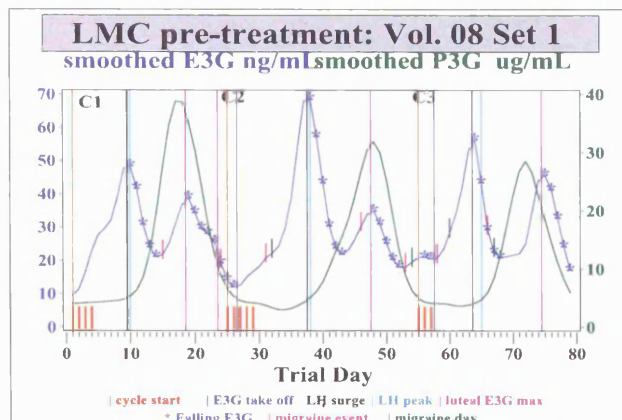
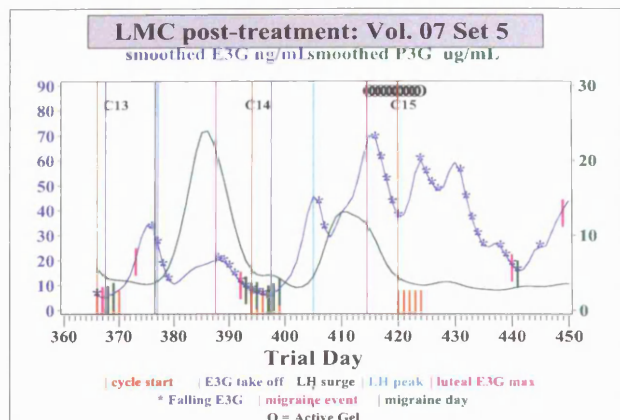
**LMC post-treatment: Vol. 07 Set 3**

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**LMC post-treatment: Vol. 07 Set 4**

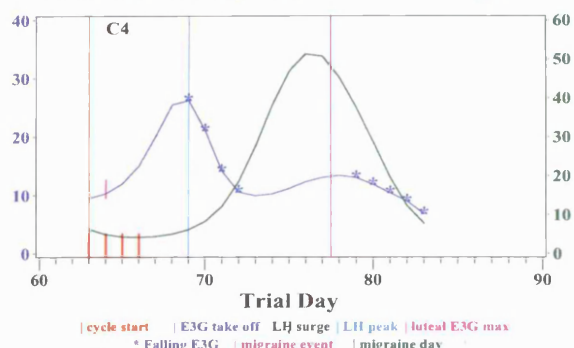
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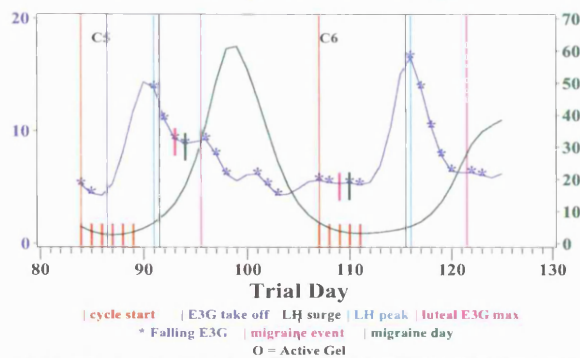
LMC pre-treatment: Vol. 09 Set 2

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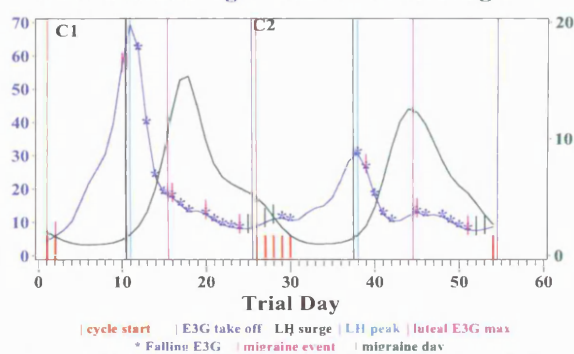
LMC post-treatment: Vol. 09 Set 2

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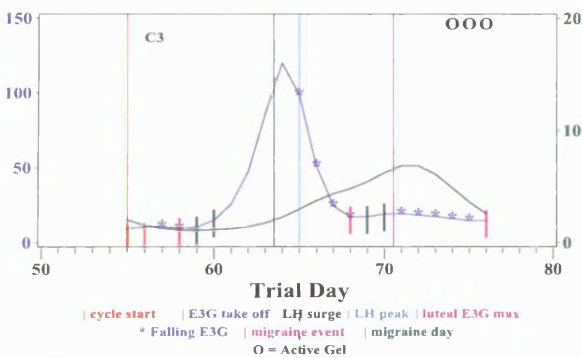
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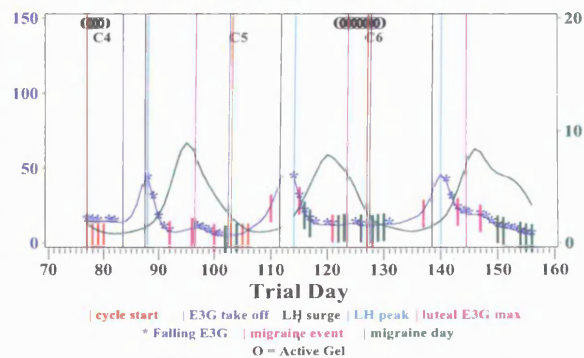
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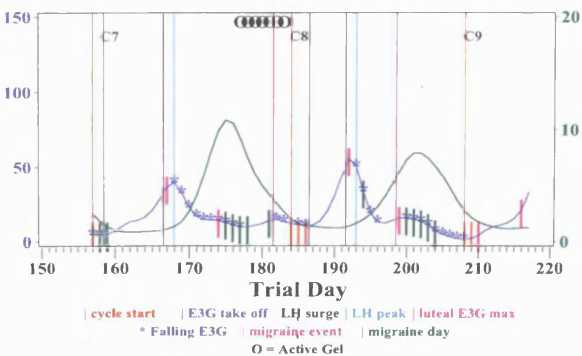
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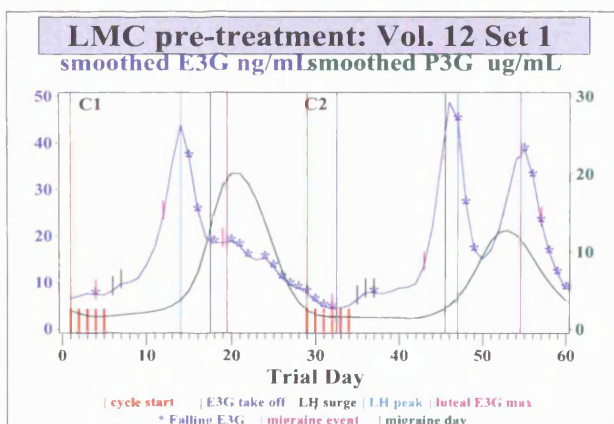
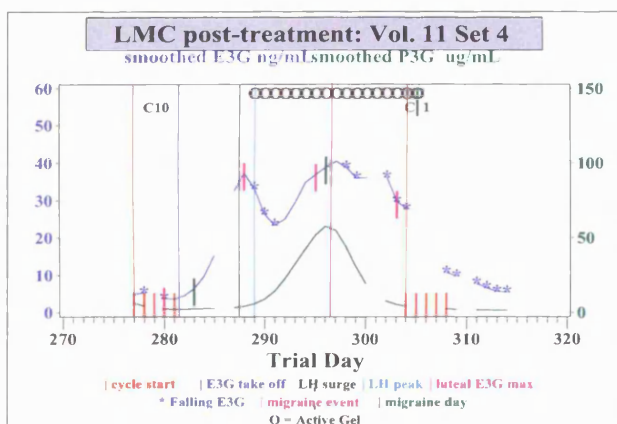
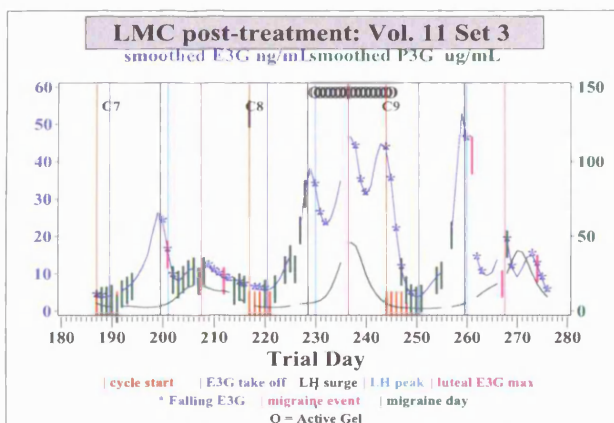
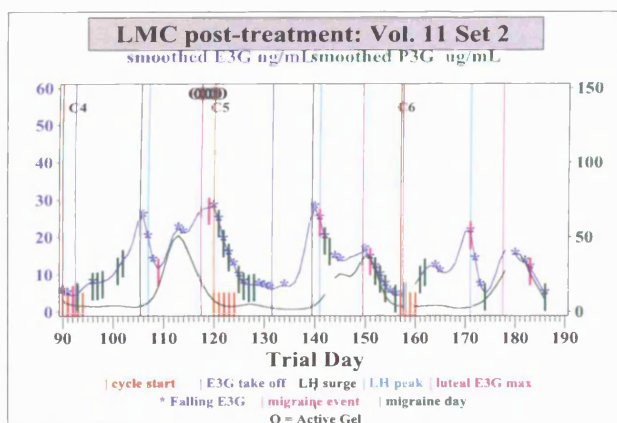
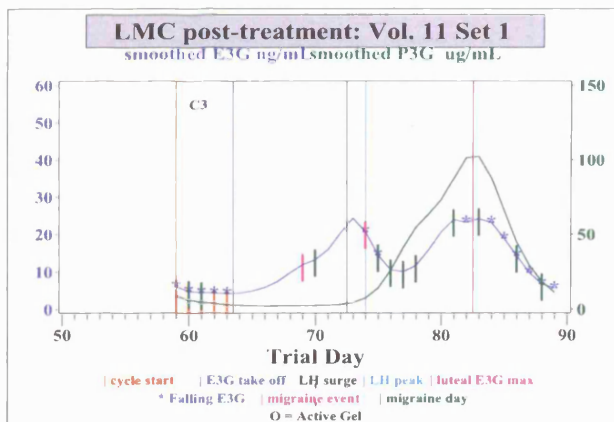
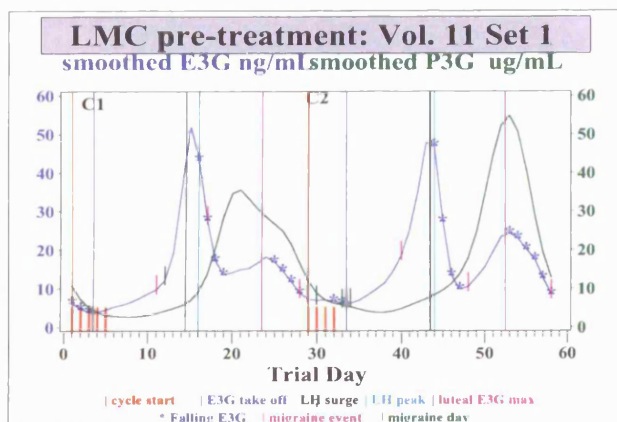
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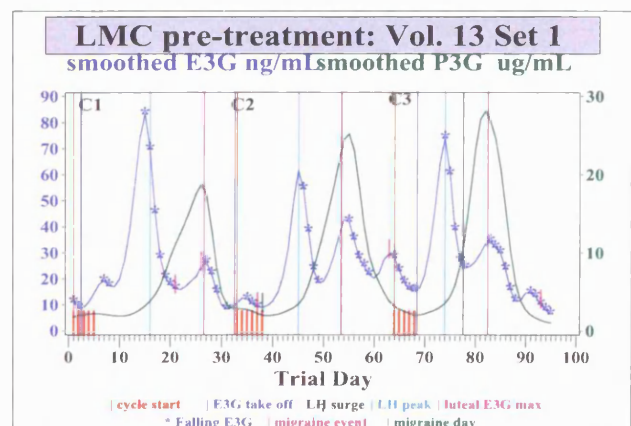
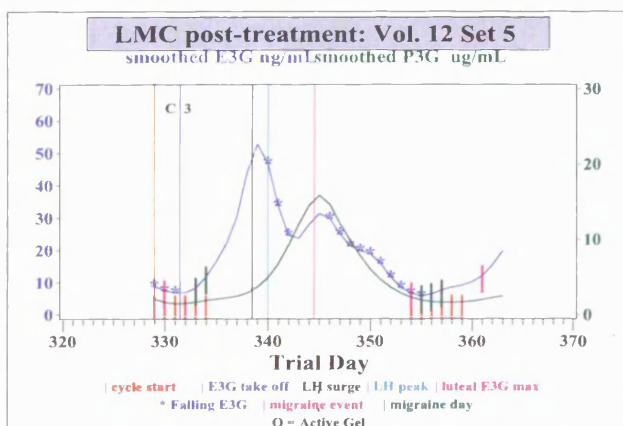
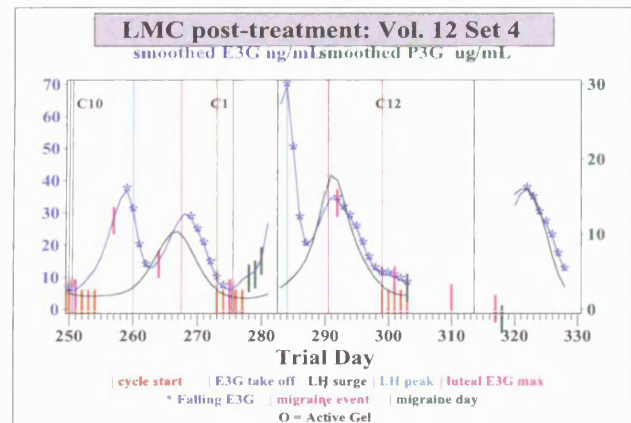
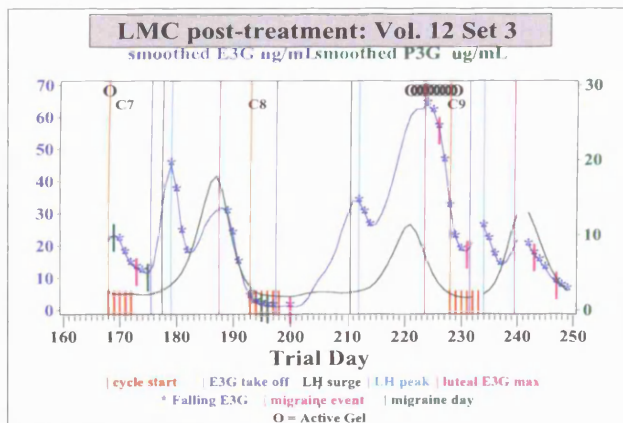
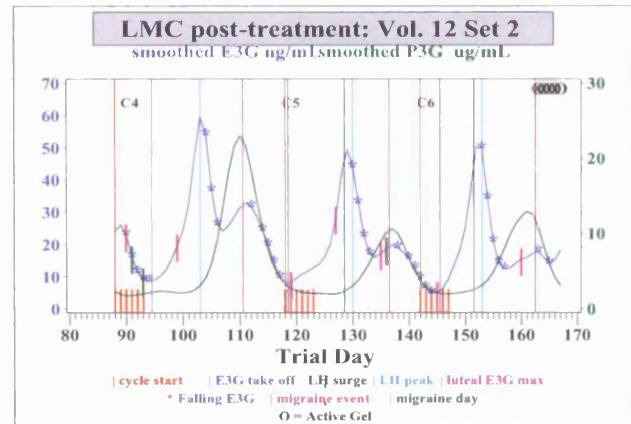
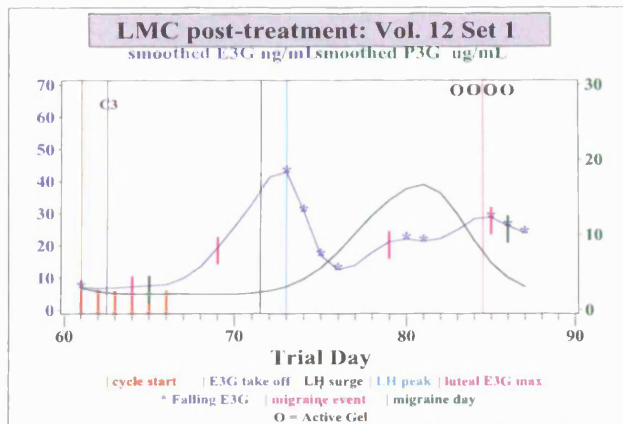


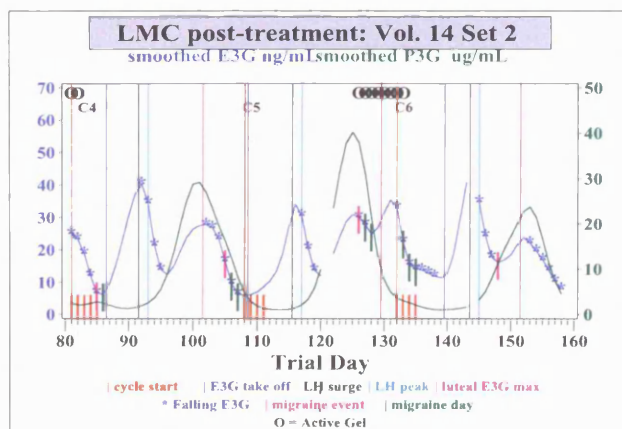
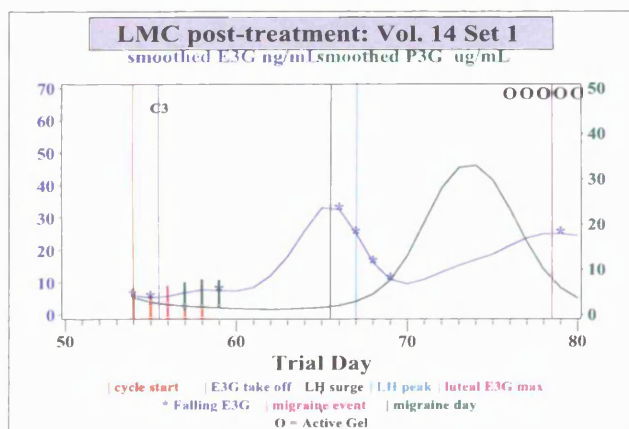
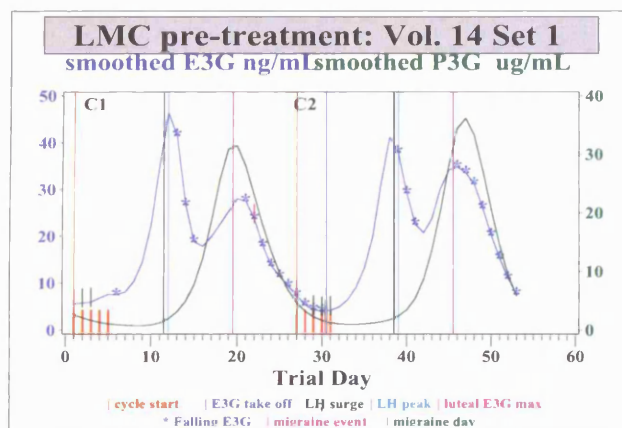
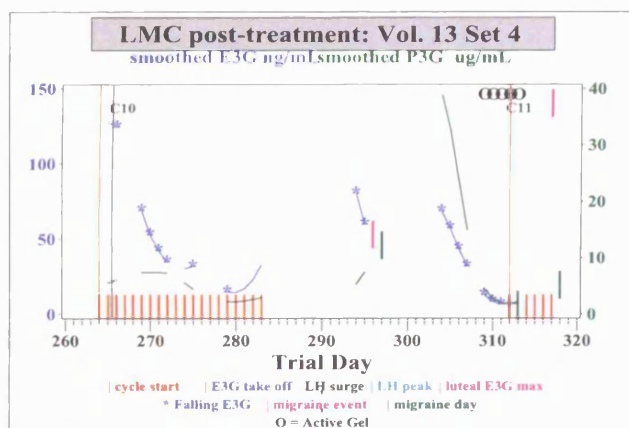
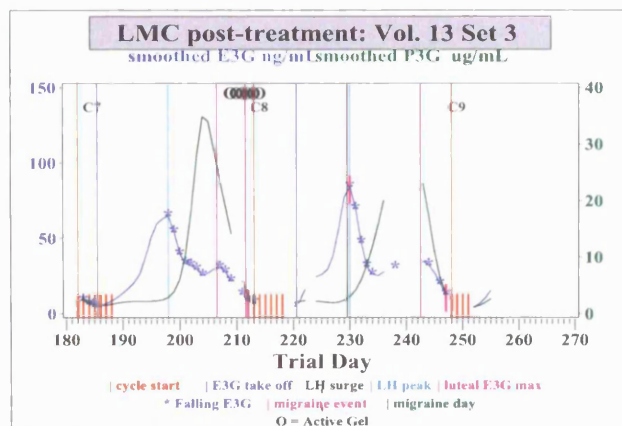
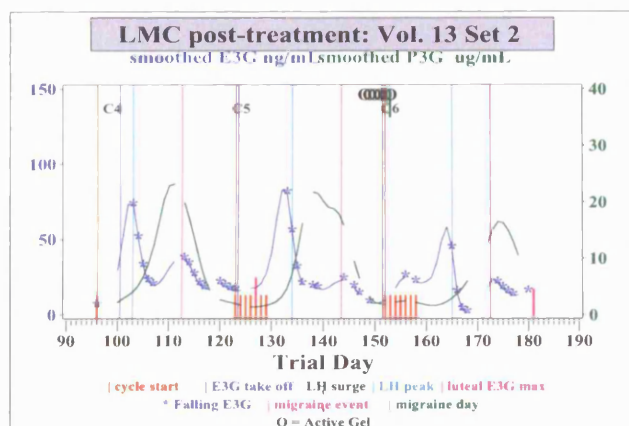
LMC post-treatment: Vol. 10 Set 3

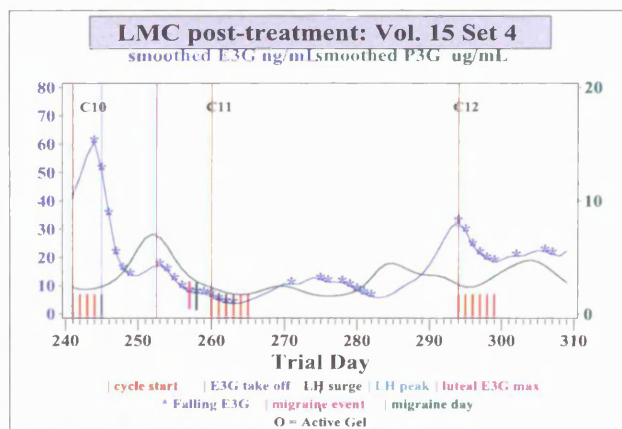
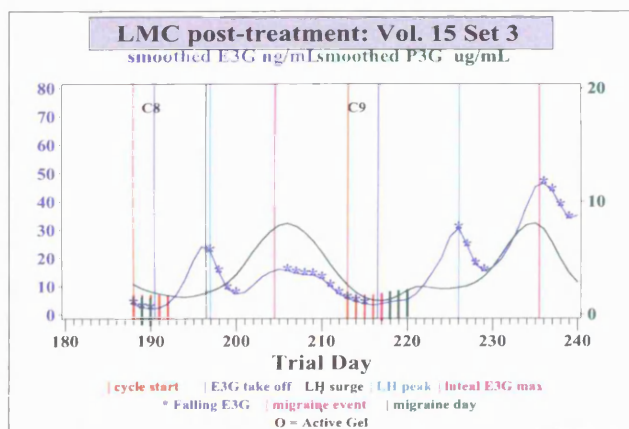
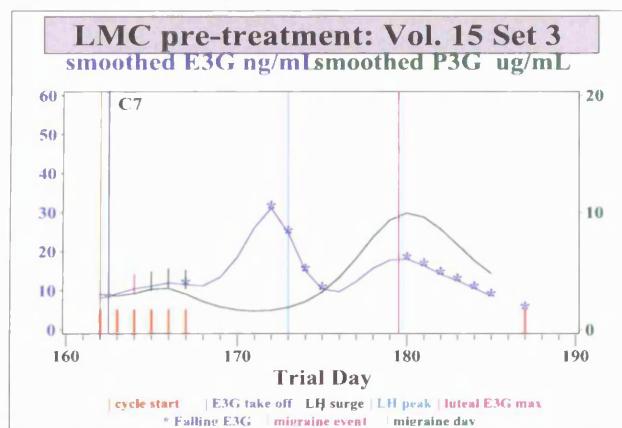
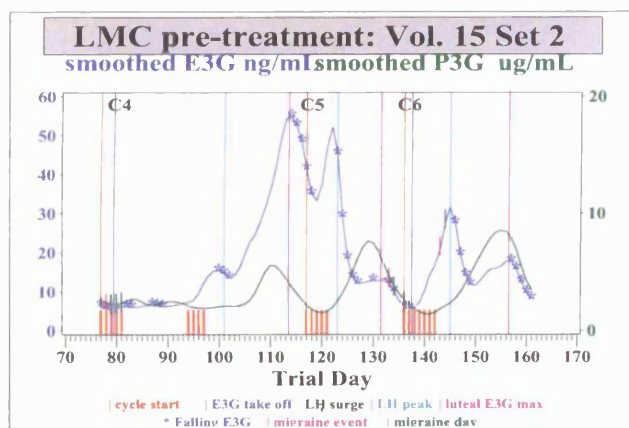
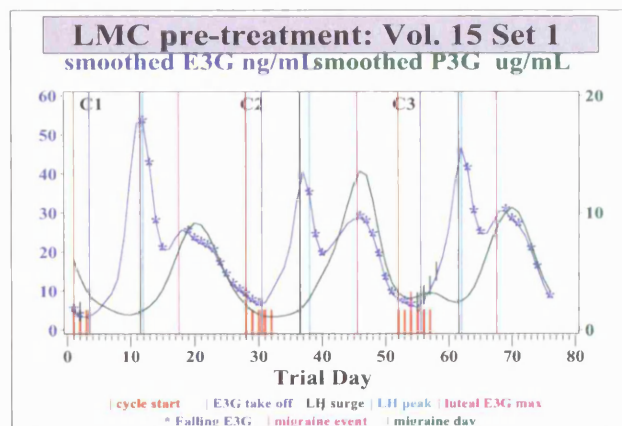
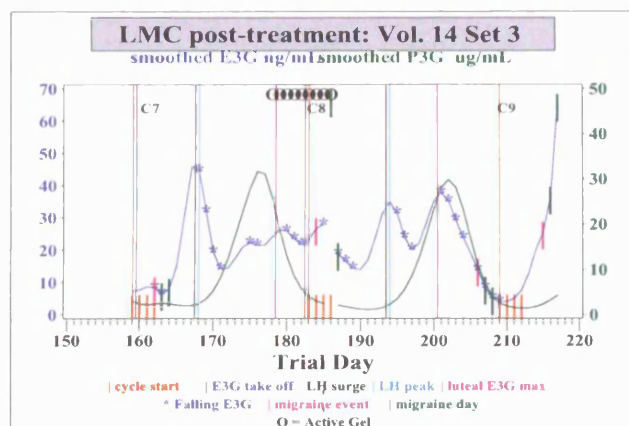
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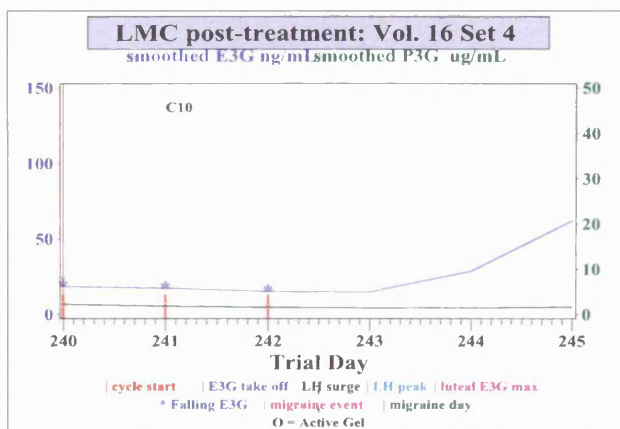
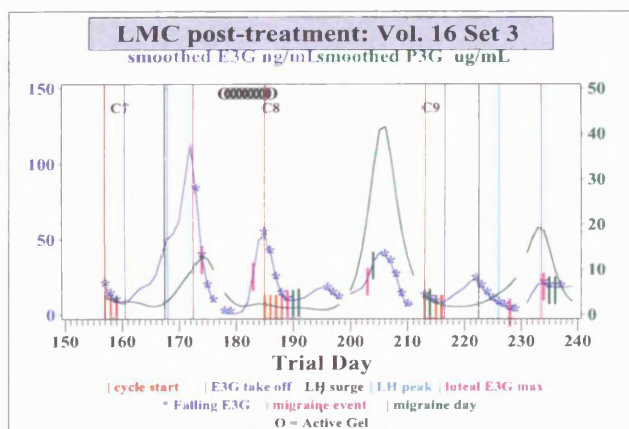
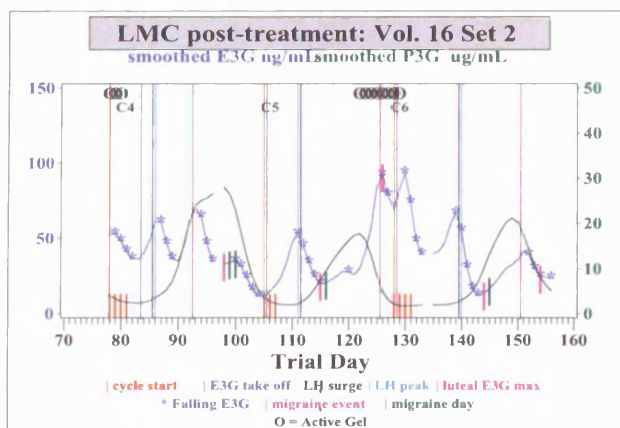
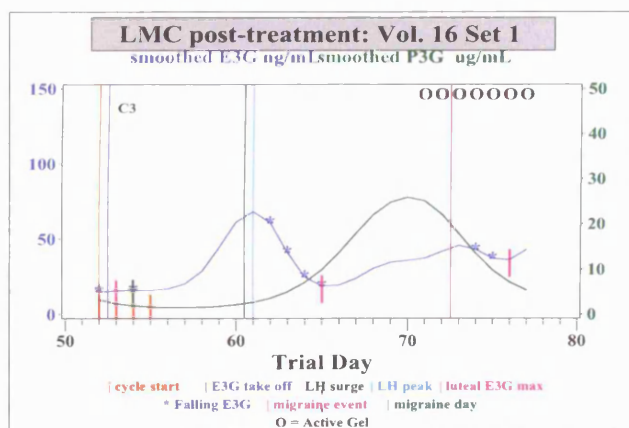
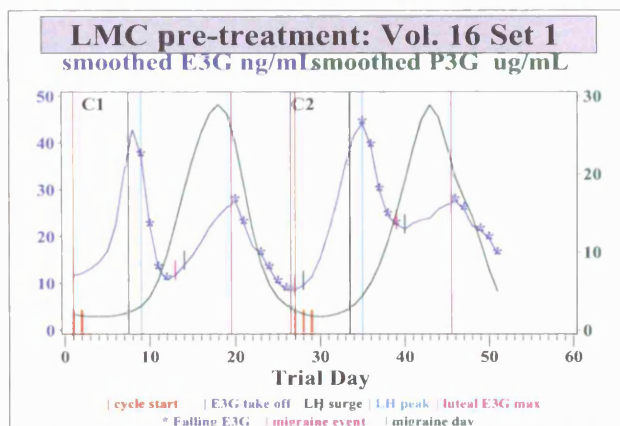
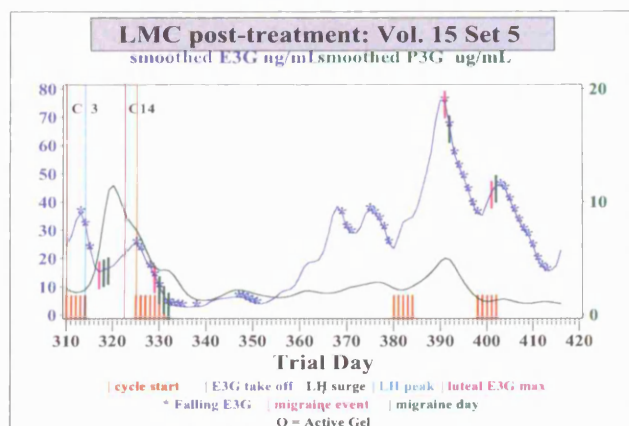


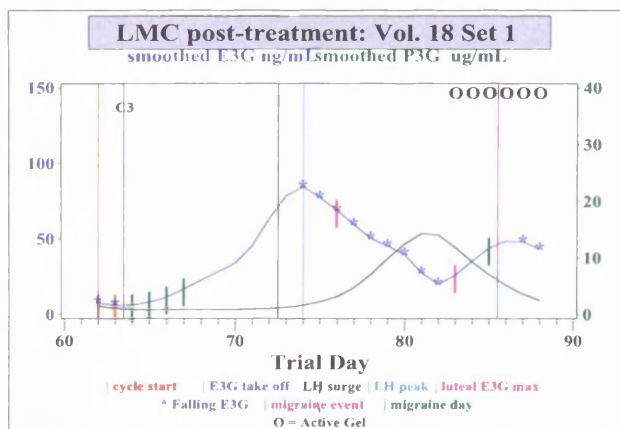
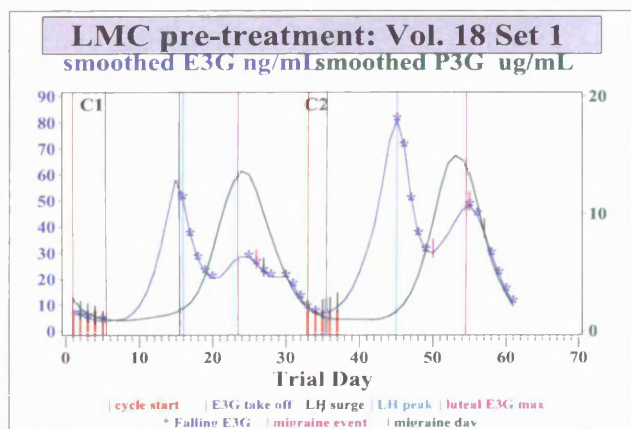
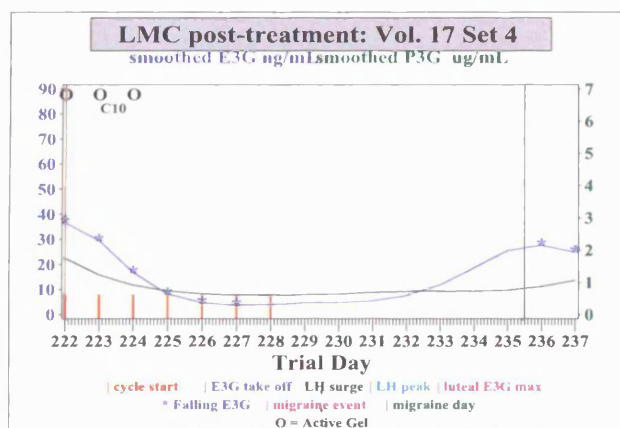
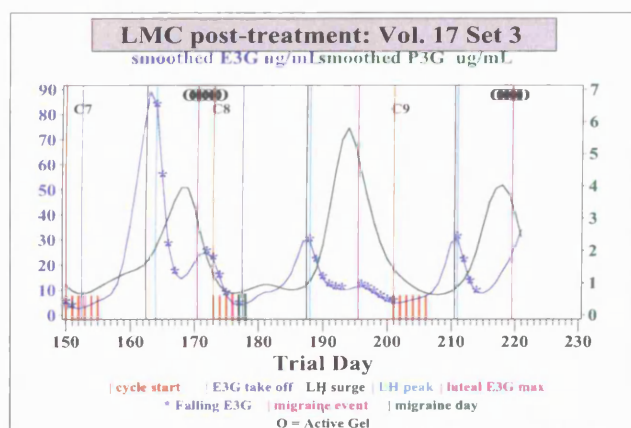
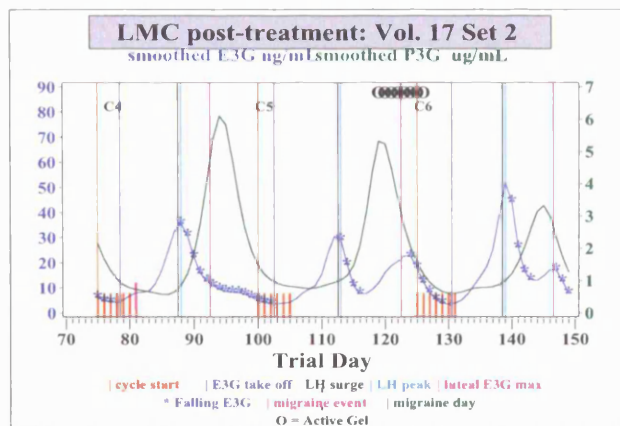
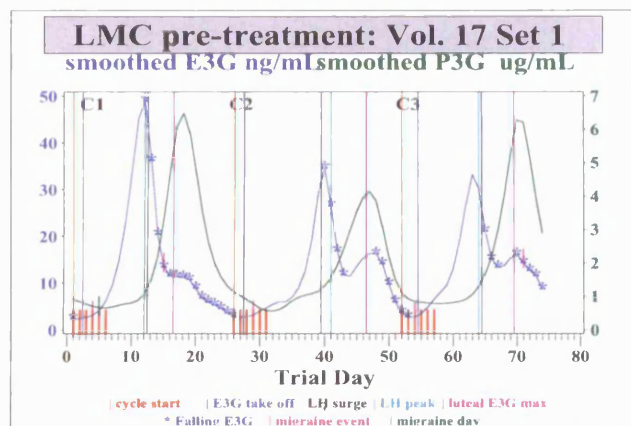


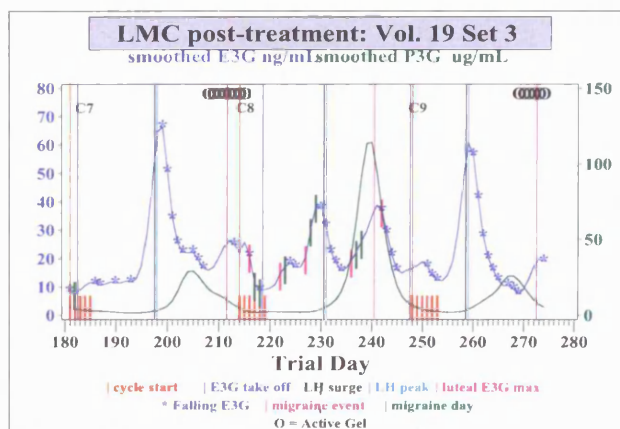
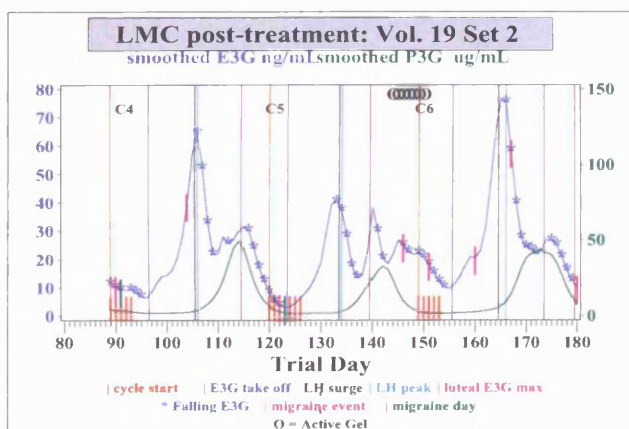
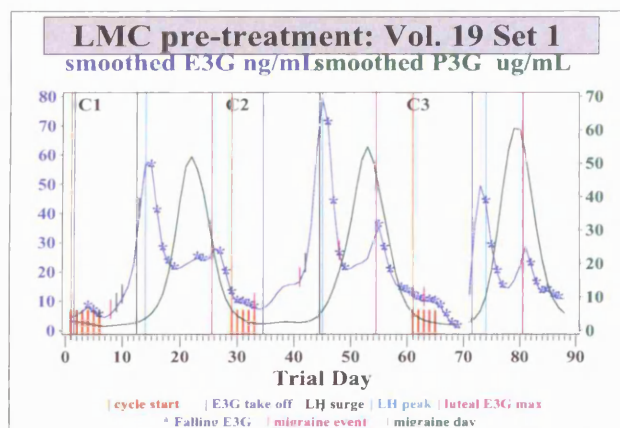
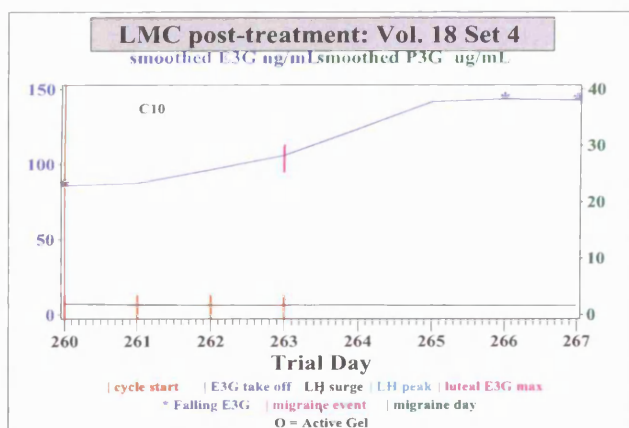
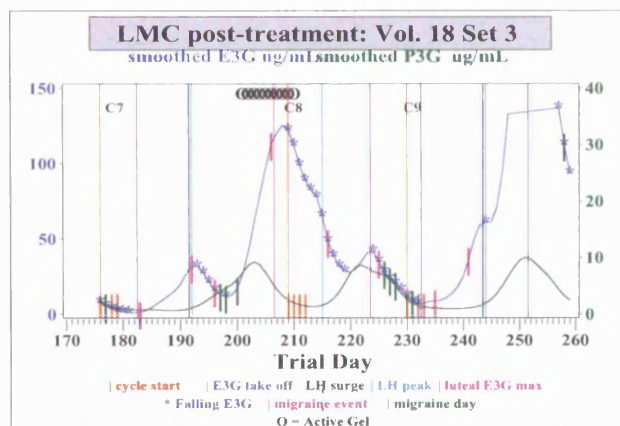
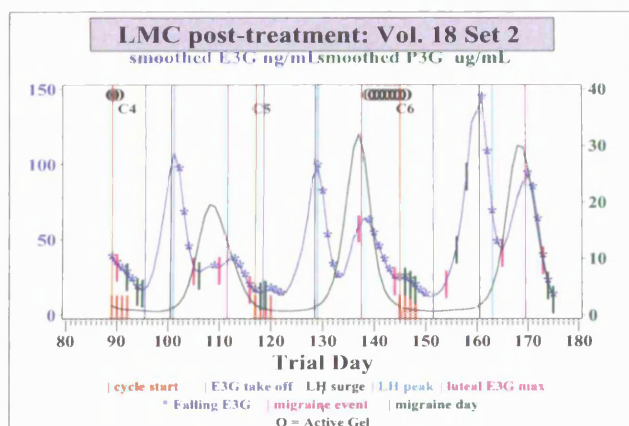


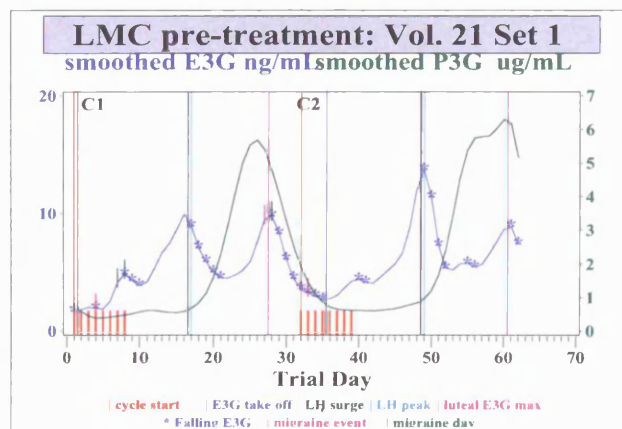
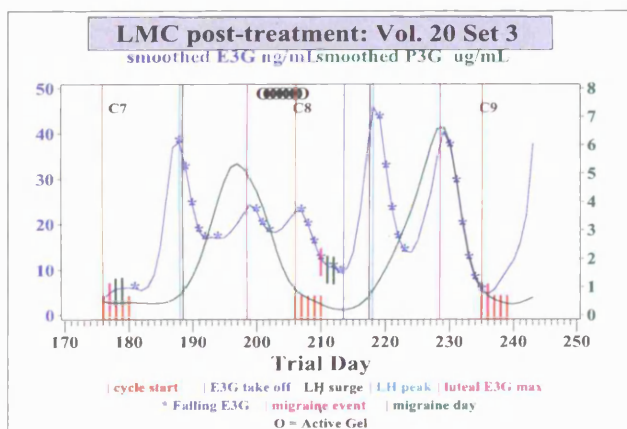
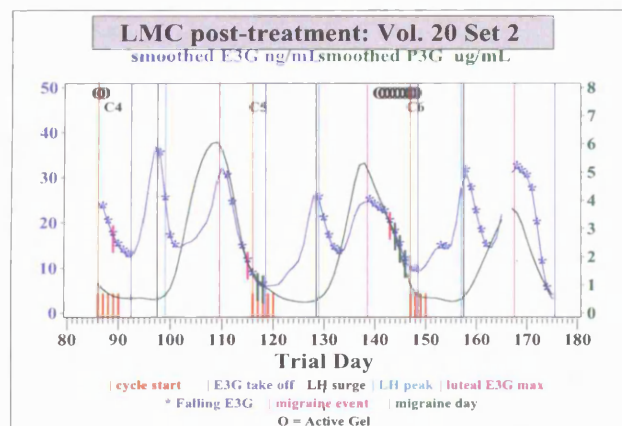
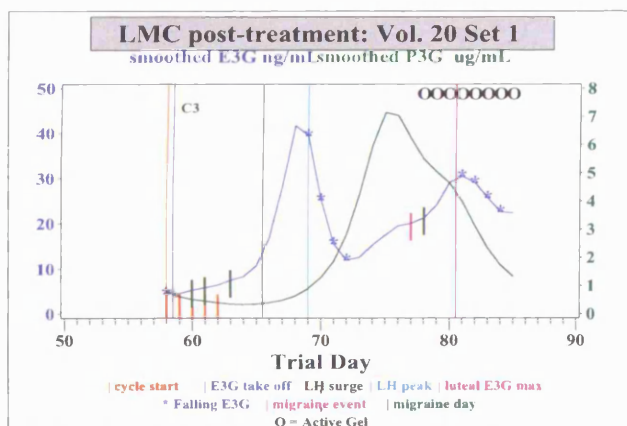
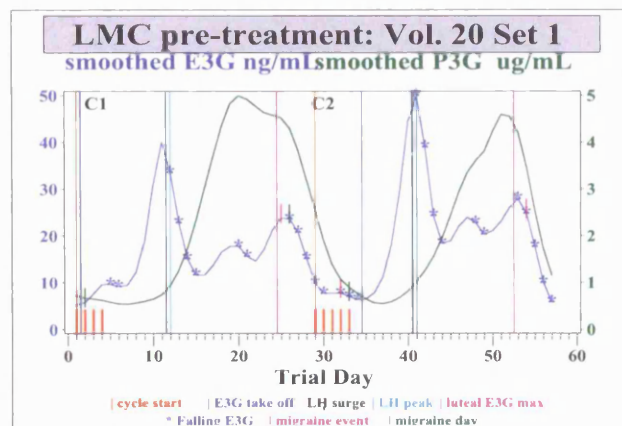
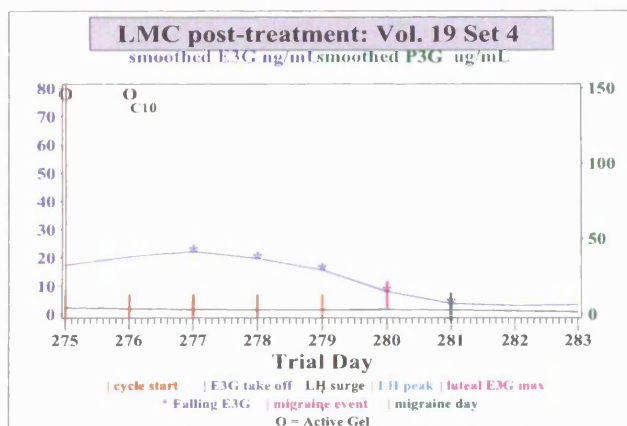


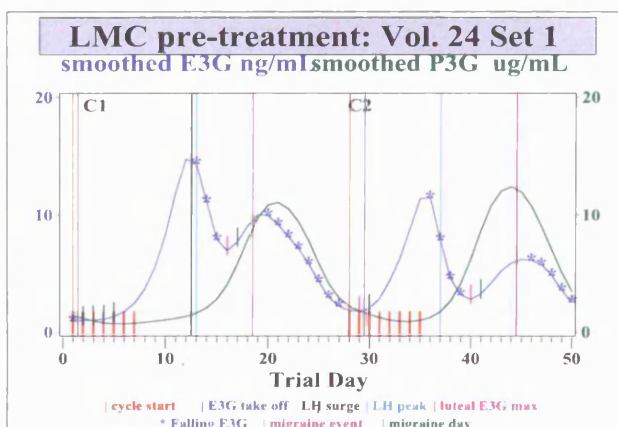
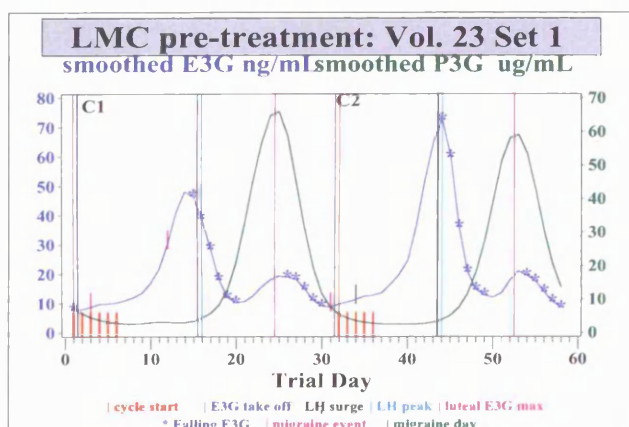
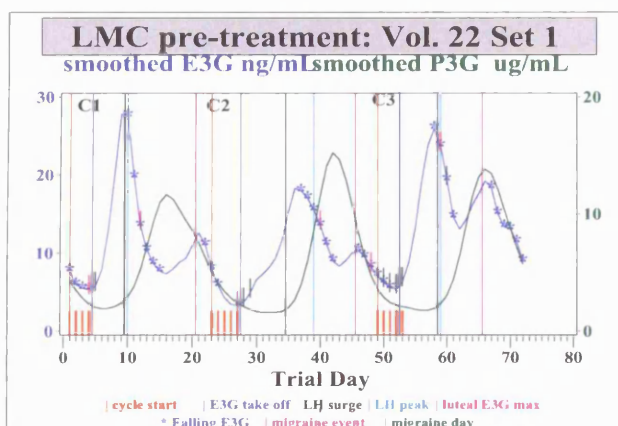
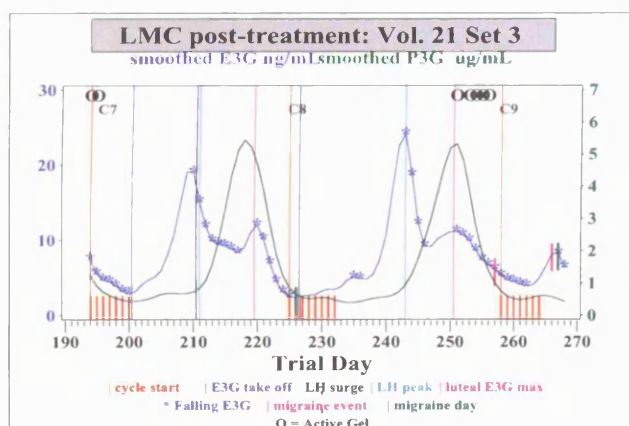
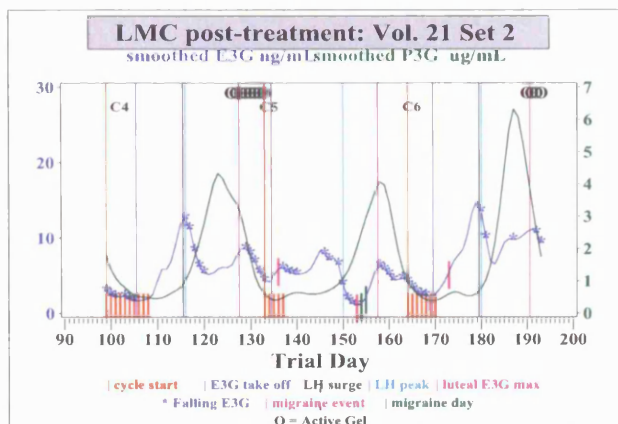
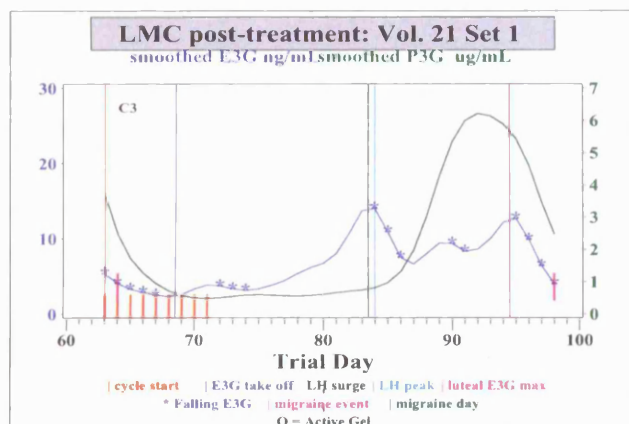


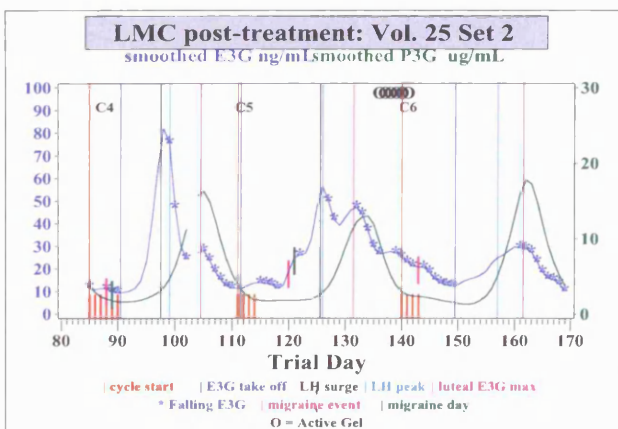
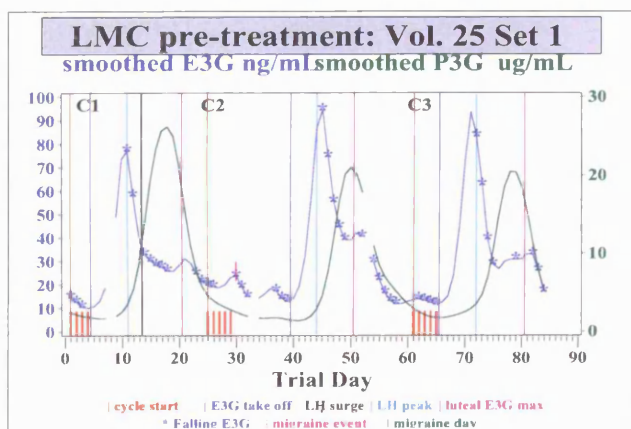
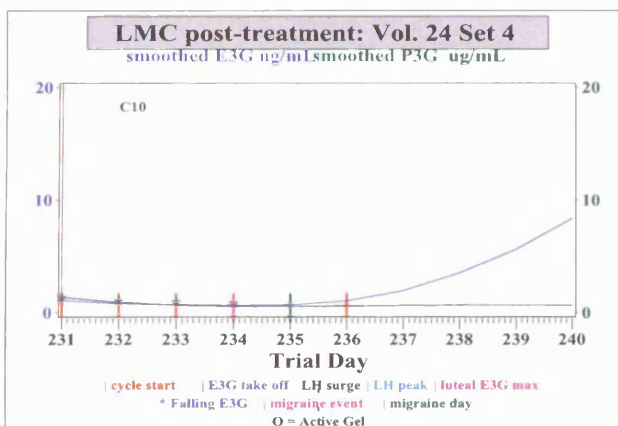
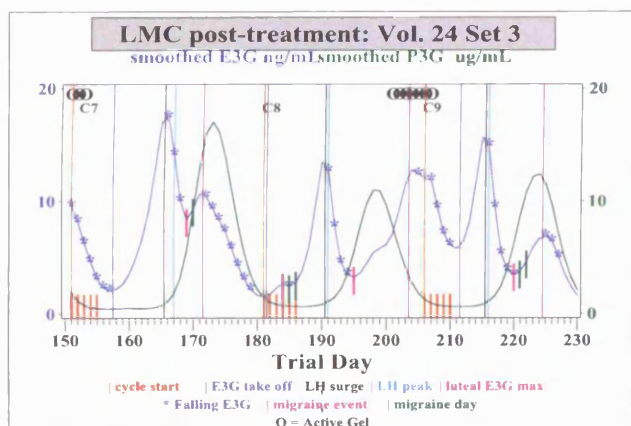
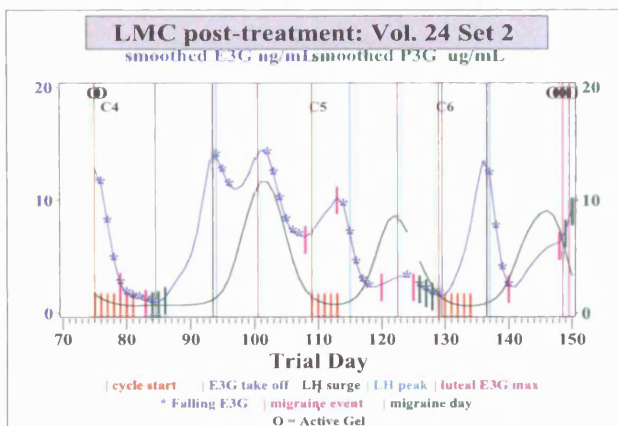
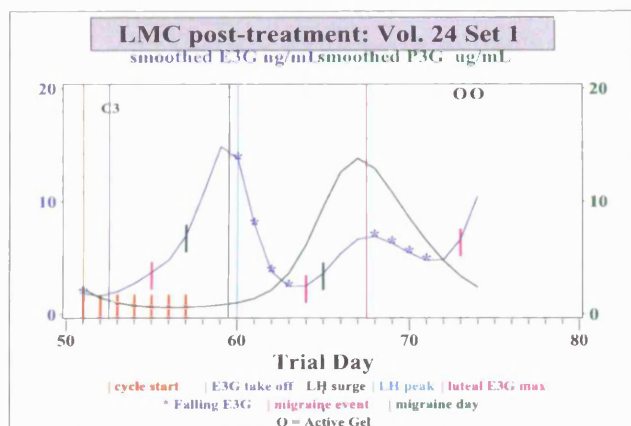


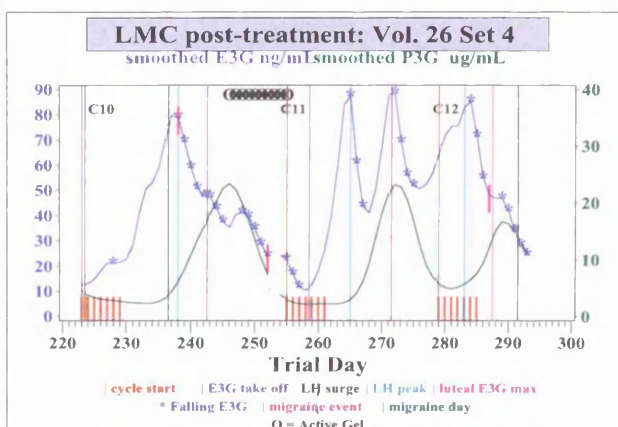
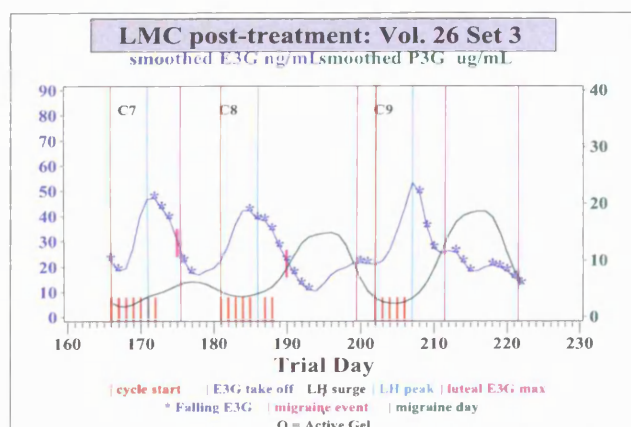
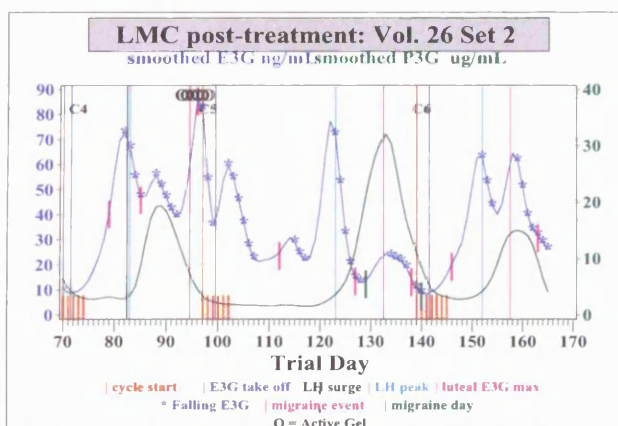
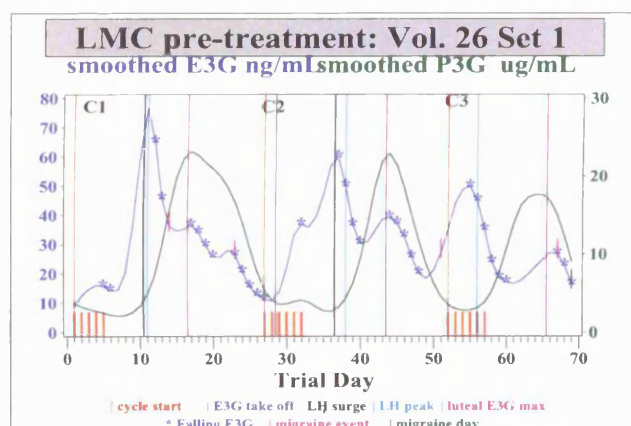
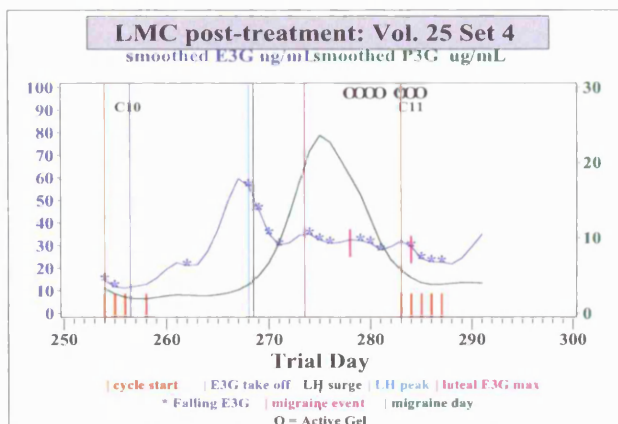
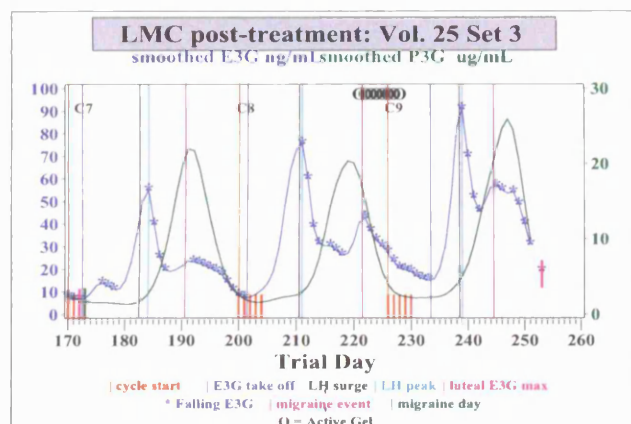


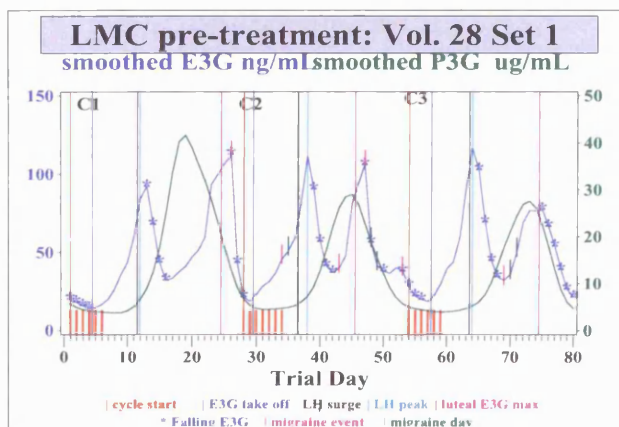
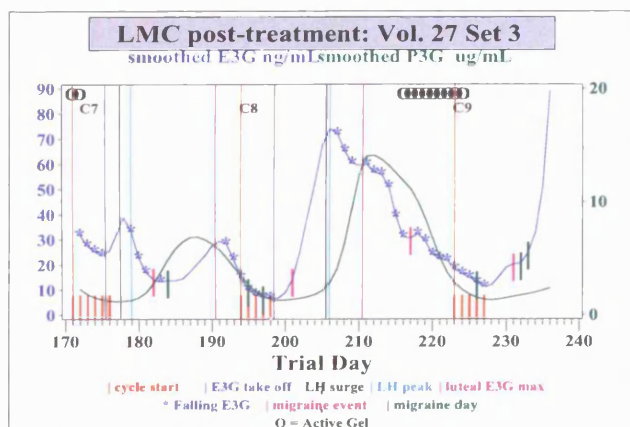
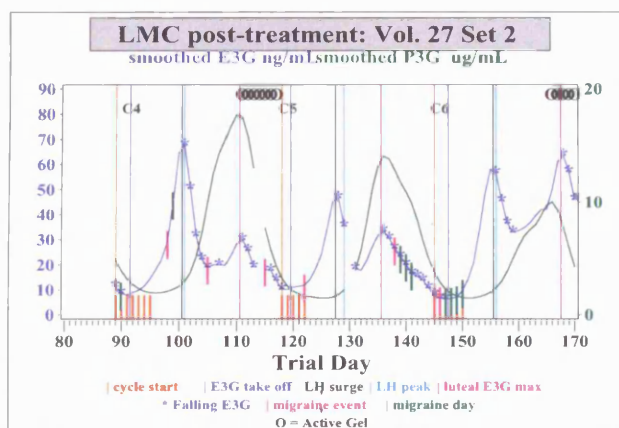
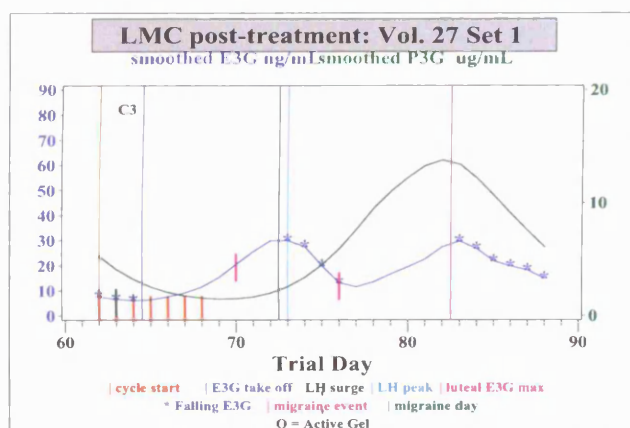
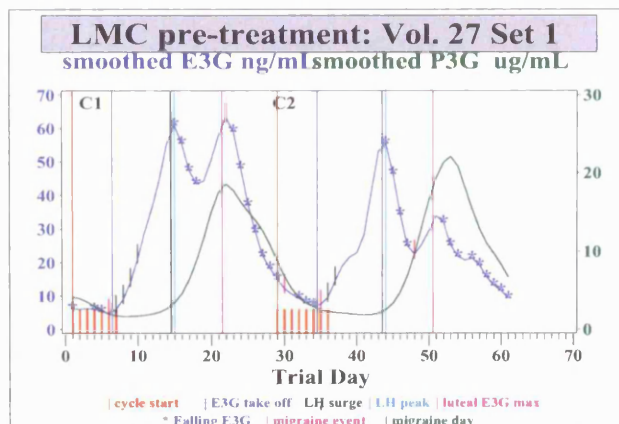
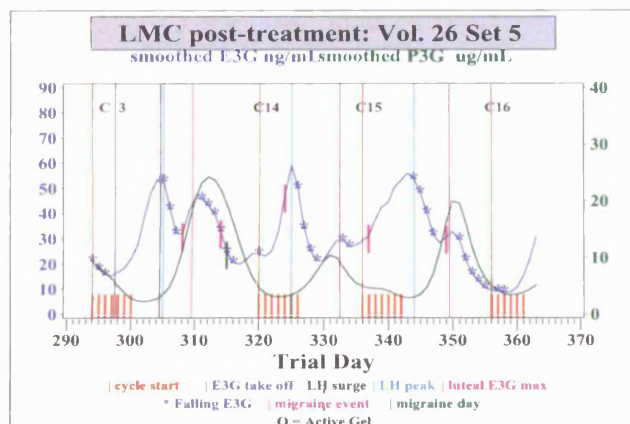


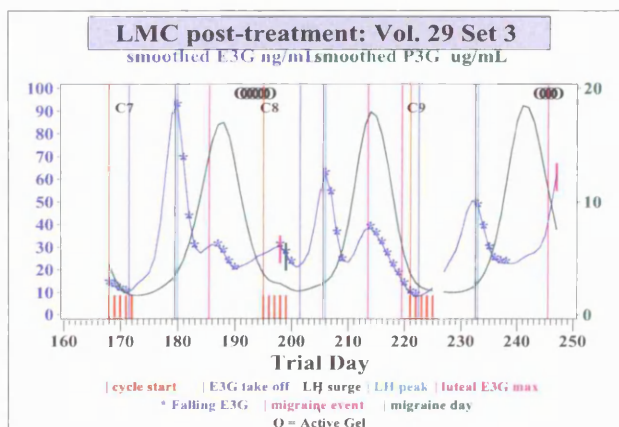
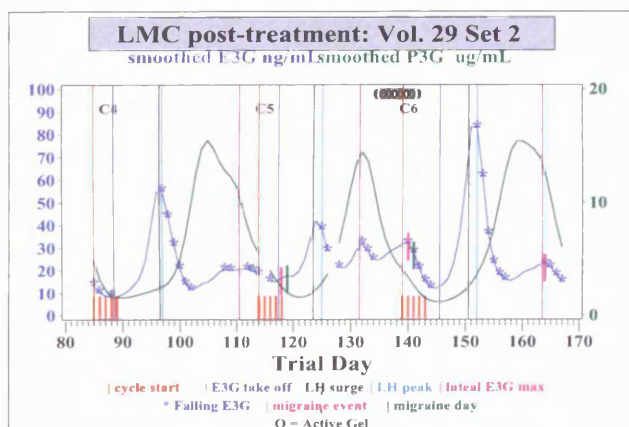
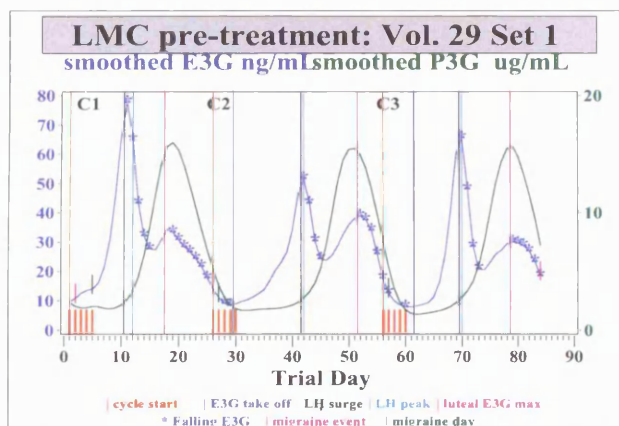
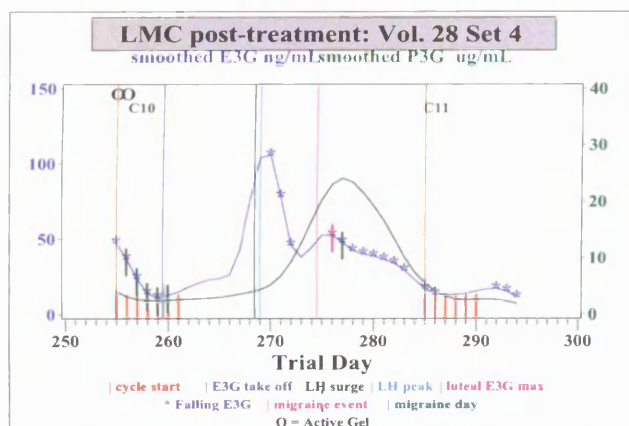
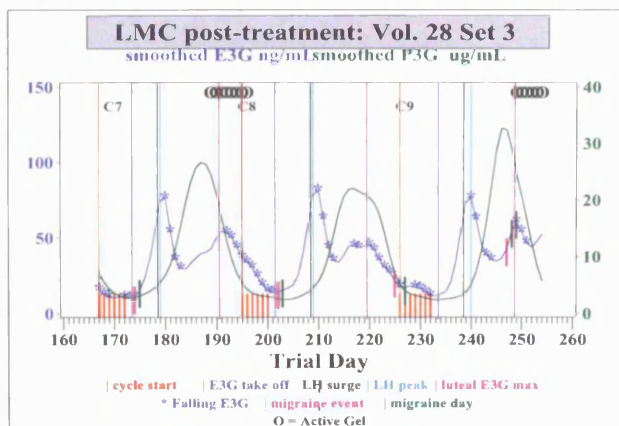
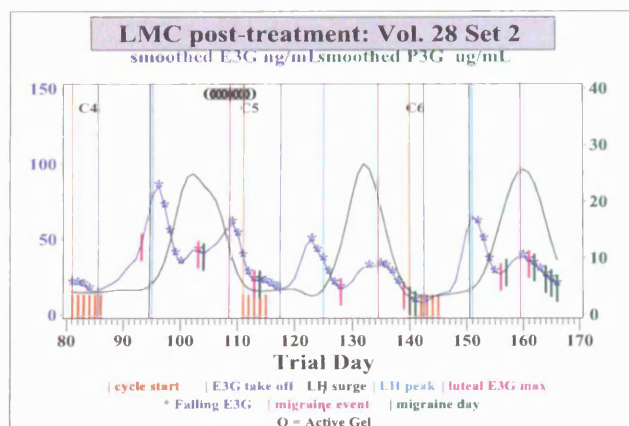


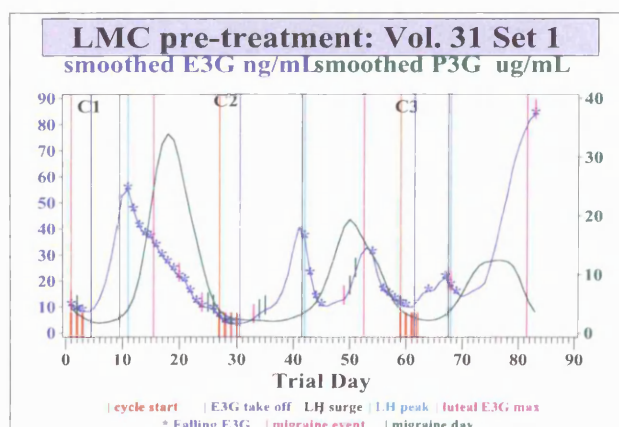
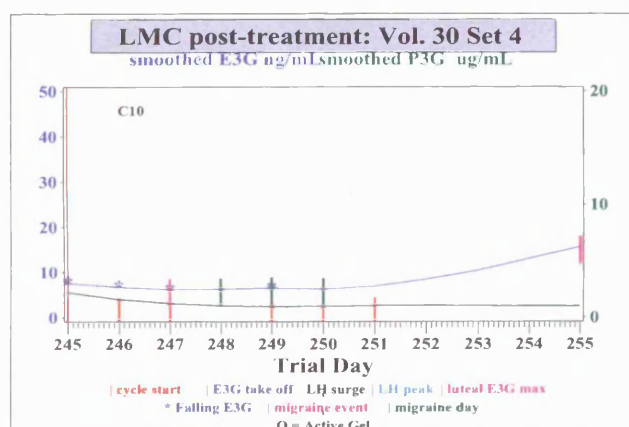
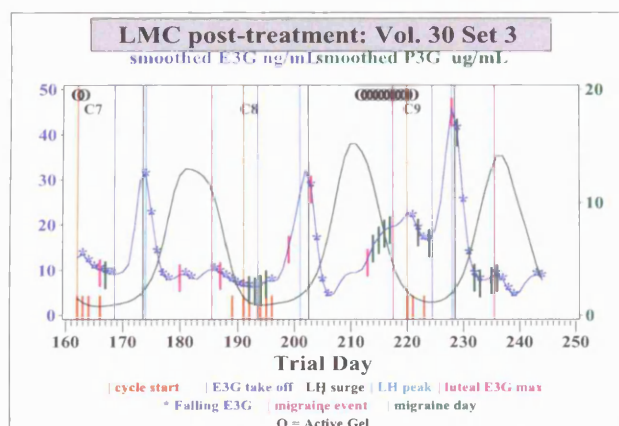
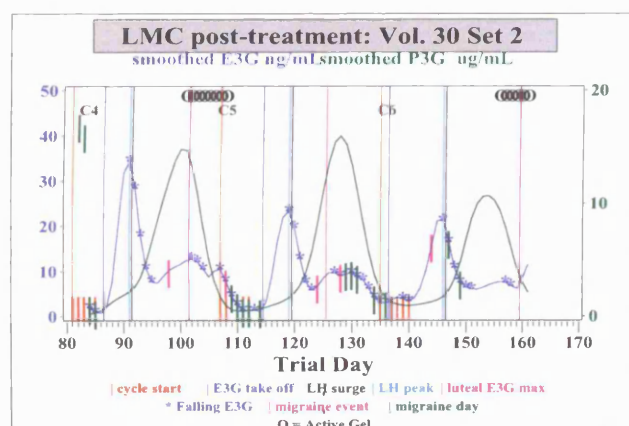
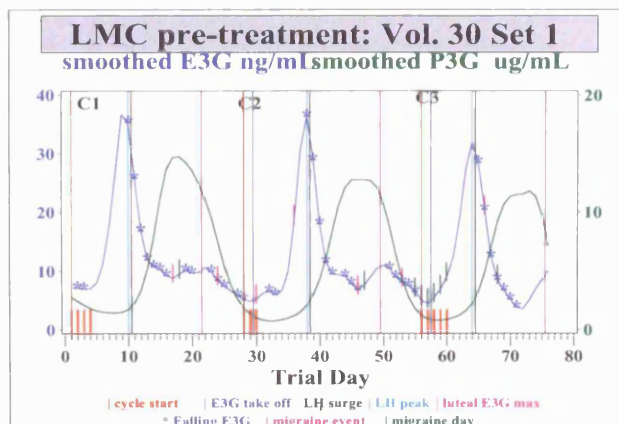
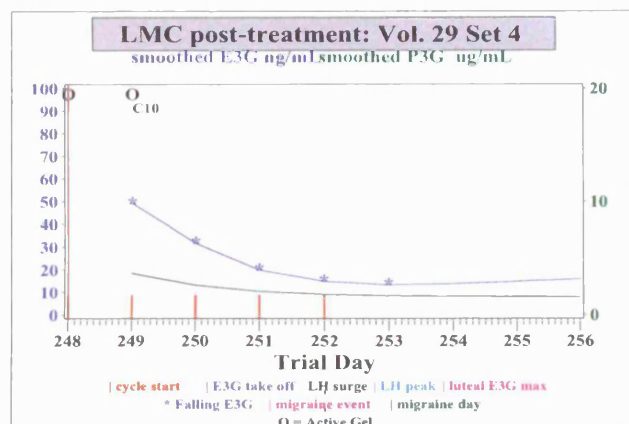


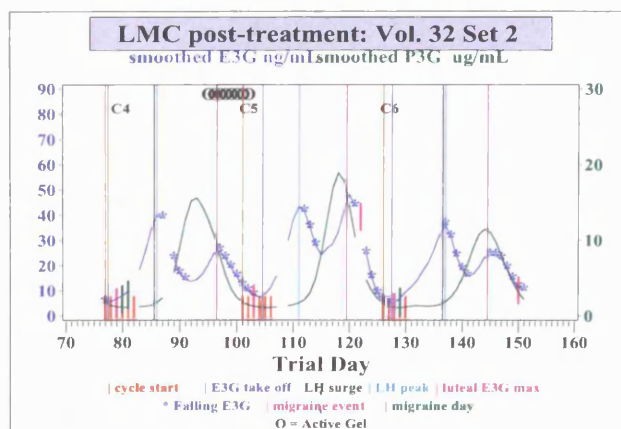
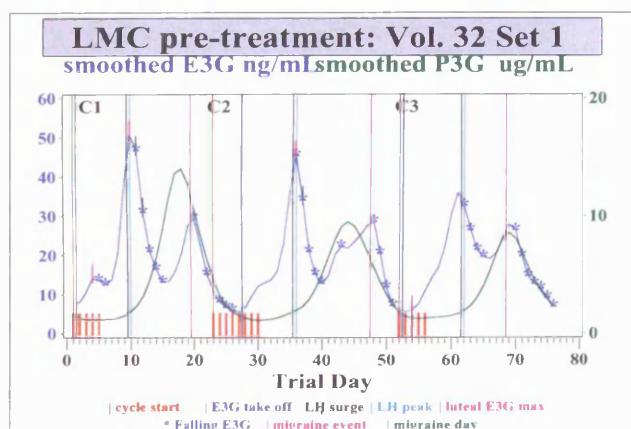
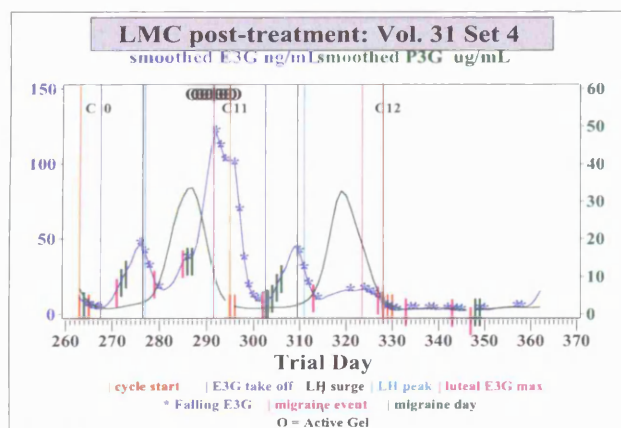
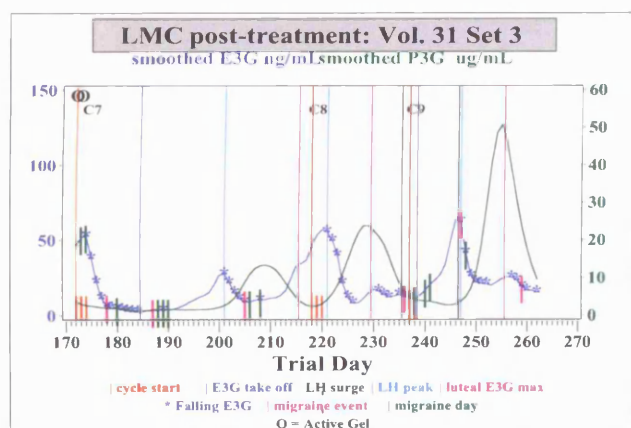
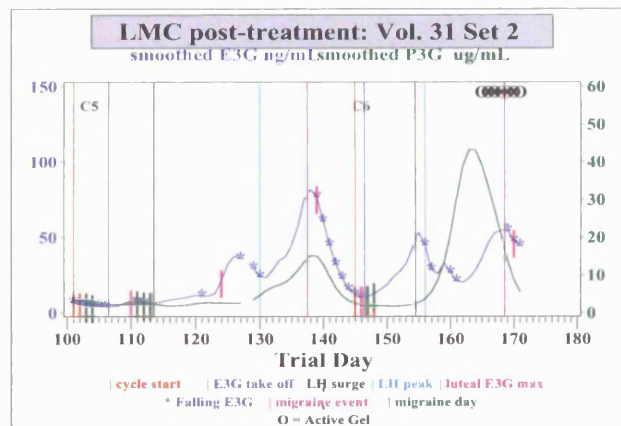
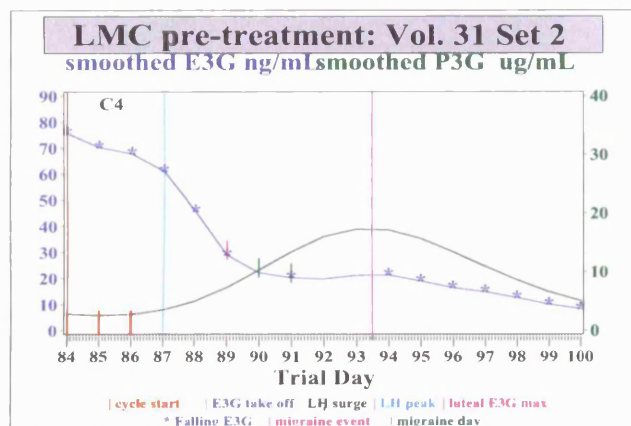


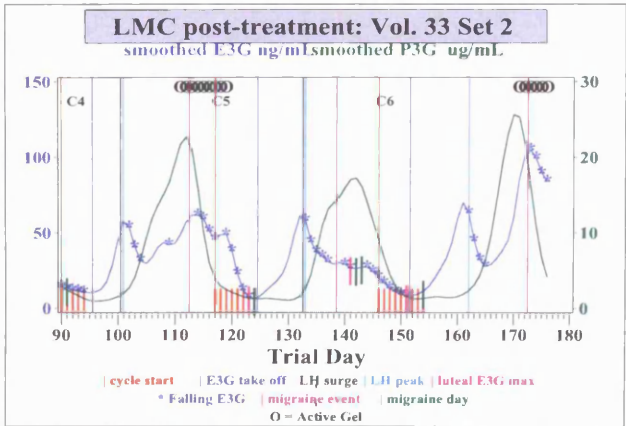
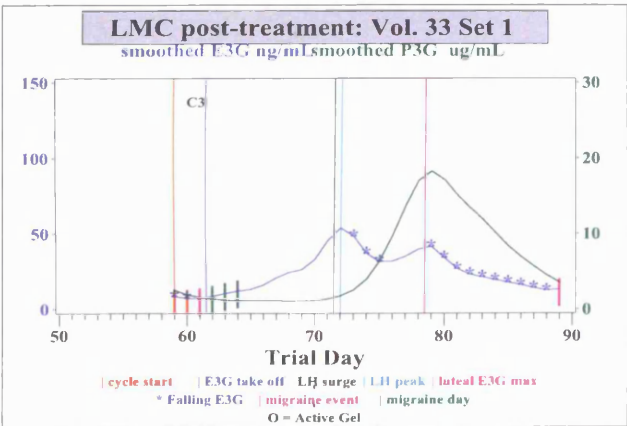
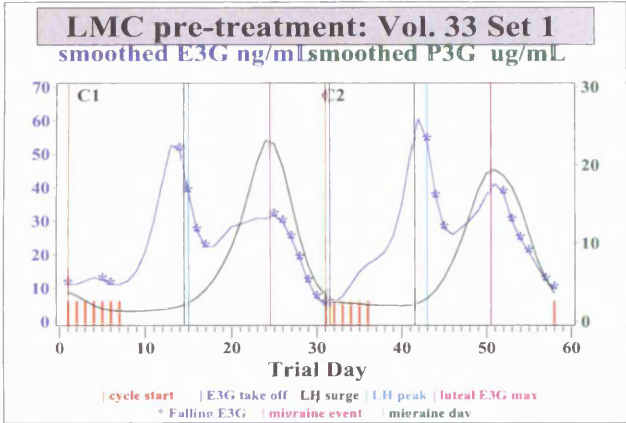
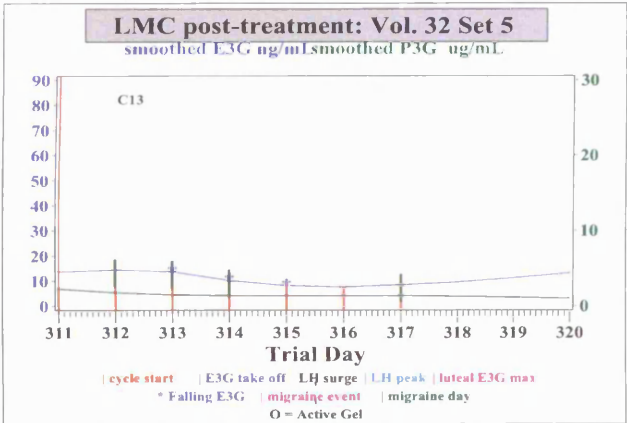
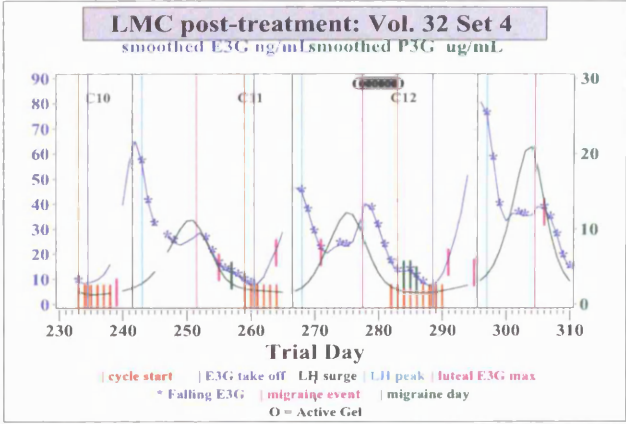
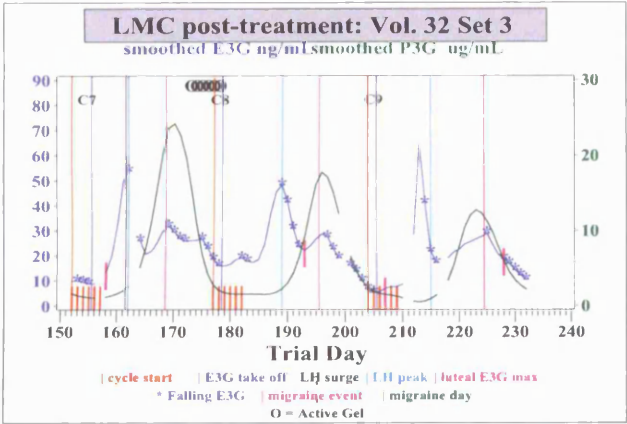


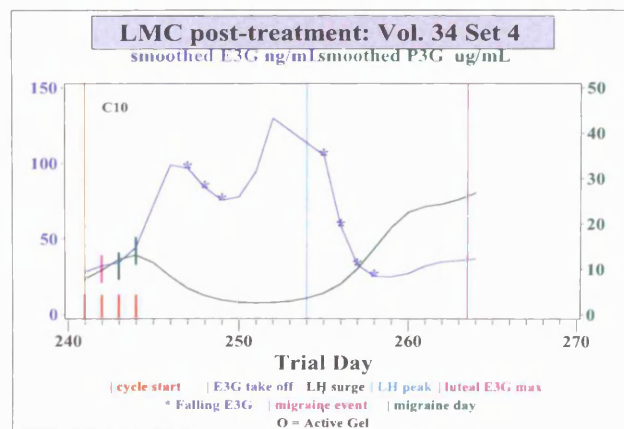
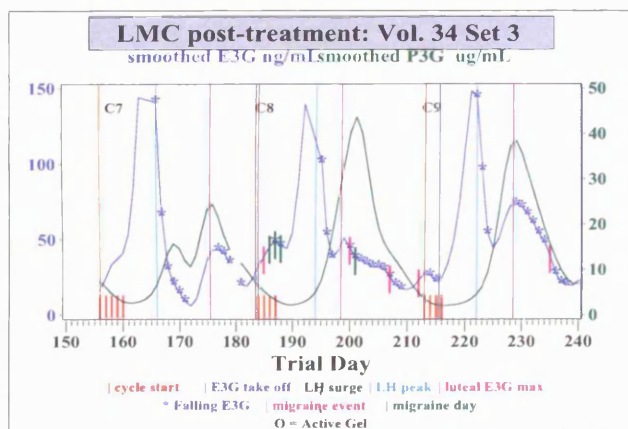
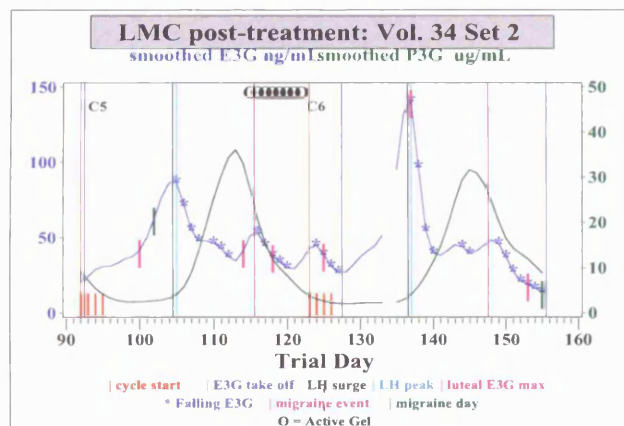
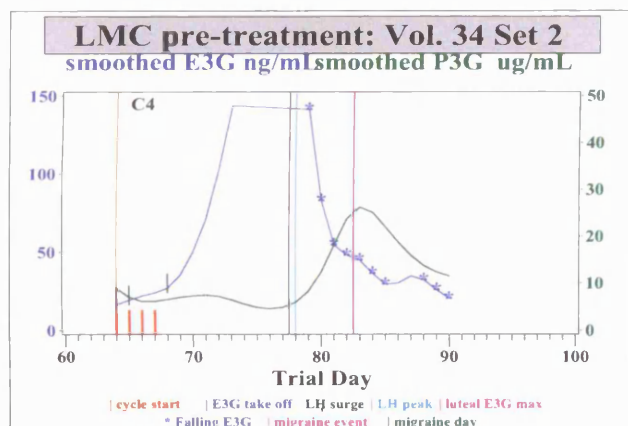
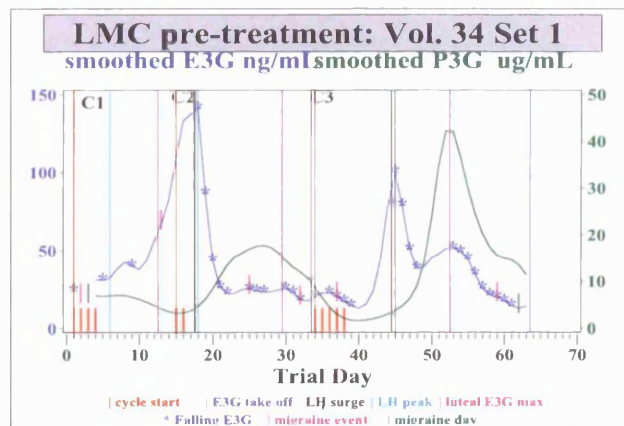
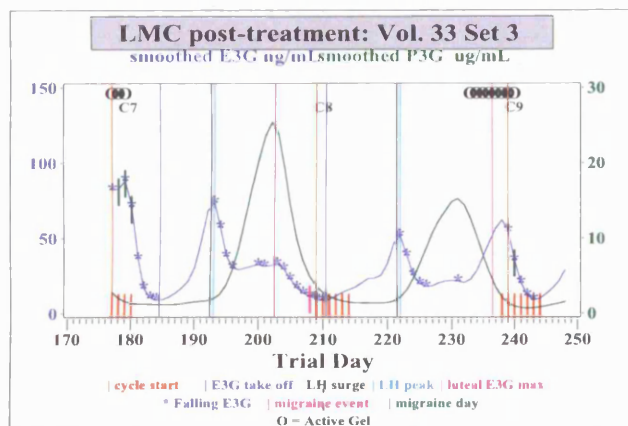




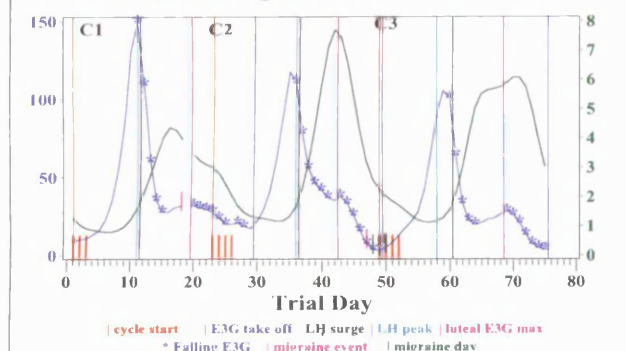




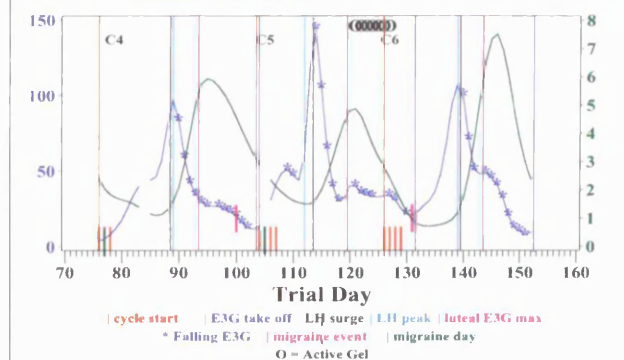




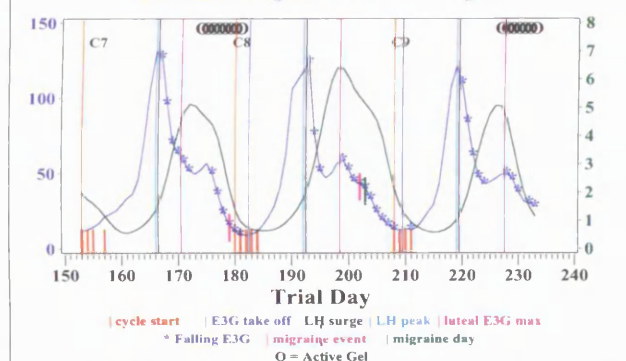
LMC pre-treatment: Vol. 35 Set 1
smoothed E3G ng/mL smoothed P3G ug/mL



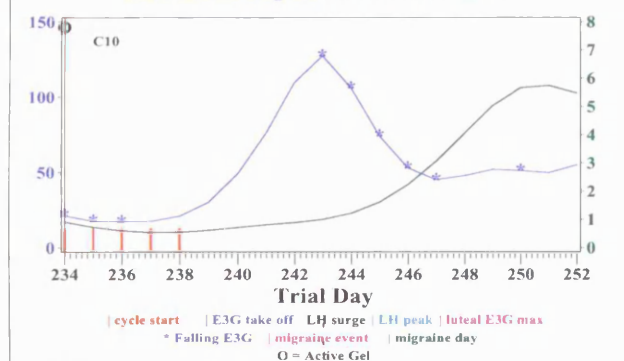
LMC post-treatment: Vol. 35 Set 2
smoothed E3G ng/mL smoothed P3G ug/mL



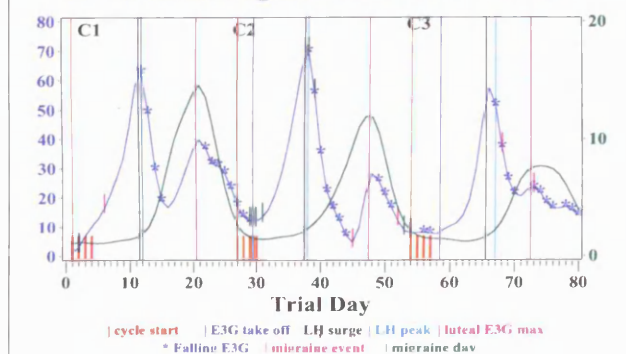
LMC post-treatment: Vol. 35 Set 3
smoothed E3G ng/mL smoothed P3G ug/mL



LMC post-treatment: Vol. 35 Set 4
smoothed E3G ng/mL smoothed P3G ug/mL



LMC pre-treatment: Vol. 36 Set 1
smoothed E3G ng/mL smoothed P3G ug/mL



LMC pre-treatment: Vol. 36 Set 2
smoothed E3G ng/mL smoothed P3G ug/mL

